

Stereoselective chemoenzymatic phytate transformations provide access to diverse inositol phosphate derivatives

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1. General Informations

All solvents and reagents (analytical grade) were obtained from commercial sources (VWR/Merck, Alfa Aesar, ChemPur, Roth, TCI, Acros, Sigma Aldrich) and used without further purifications, if not stated otherwise.

Dry solvents were either obtained from commercial sources (VWR/Merck, Alfa Aesar, ChemPur, Roth, TCI, Acros, Sigma Aldrich) or purified on a *Braun Solvent Purification System 800* and stored over activated molecular sieves (3Å) und an argon atmosphere.

Flame dried glass ware was used for all reactions. If moisture sensitive reagents were applied, the reactions were conducted under an atmosphere of Argon.

Strong Anion Exchange (SAX) chromatography was performed on an ÄKTA™ pure system. Samples were eluted from the solid phase (Q-sepharose fast flow (Cytiva) columns) using a gradient of NaClO₄ (1 M), NH₄HCO₃ (1 M, pH = 8) or NH₄COOH (1 M, pH = 7).

A PuriFlash® 5.125 (Interchim®) was used for reverse phase medium pressure liquid chromatography (RP-MPLC), using prepacked columns, obtained from Interchim®, as stationary phase.

Christ Alpha 1-4 LDplus or *Alpha 1-2 LDplus* freeze drier were used for lyophilisation of samples. *Bruker Avance III HD 300 MHz*, *Avance 400 MHz* or *Avance III HD 500 MHz* spectrometers were used measure nuclear magnetic resonante (NMR) spectra. Deuterated solvents were purchased from commercial suppliers (Eurisotop, Deutero), used without further purifications and stored over activated molecular sieves (3Å). *MestreNova 12.01* was used to analyse the resulting spectra, referenced to the respective deuterated solvent peaks (for ¹H-NMR: CDCl₃: δ = 7.26 ppm, DMSO-d₆: δ = 2.50, MeCN-d₃: δ = 1.94, DMF-d₇: δ = 2.92 ppm, D₂O: δ = 4.79 ppm; for ³¹C-NMR: CDCl₃: δ = 77.16 ppm, DMSO-d₆: δ = 39.52, MeCN-d₃: δ = 1.32 ppm, DMF-d₇: δ = 34.9 ppm.).¹

1.1. Capillary Electrophoresis

Capillary electrophoresis (CE) was measured on a fused silica capillary (100 cm, 50 μ m, internal diameter and 365 μ m outer diameter) on an *Agilent 7100* capillary electrophoresis system coupled to an *Agilent 6520* Q-TOF- or *Agilent 6495C* QQQ-mass spectrometer, equipped with an *Agilent Jet Stream* electrospray ionization source. Data acquisition was performed with *Agilent OpenLAB CDS Chemstation 2.3.53*, the resulting data was analyzed with *Agilent MassHunter Workstation Version B.08.00*. The capillary was flushed with NaOH (1 N, 10 min) and water (10 min) for equilibration. The capillary was equilibrated with back ground electrolyte (BGE) (10 min) prior to every measurement. The following BGE were used: NH₄OAc (35 mM, pH = 9.9, titrated with NH₃) and NH₄OAc (40 mM, pH = 9.1, titrated with NH₃). Isopropanol:water (1:1), spiked with mass references (TFA anion, [M-H]⁻: 1112.9855 and HP-0921, [M-H+CH₃COOH]⁻: 980.0163) for qTOF measurements, was deployed as sheath liquid using an isocratic pump with constant flow rate (1.5 μ L/min).

Samples were applied *via* pressure (100 mbar, 10-15 s, 20-30 μ L), followed by BGE injection (50 mbar, 2 s) and voltage (30 kV) was applied over the capillary to establish a constant current (23 μ A). The following source parameters (Supplementary Table 1) were used for measurements in negative ionization mode.

Analytes were identified based on exact m/z ratios, for qTOF measurements and based on characteristic fragmentation patterns (Supplementary Table 2), for QQQ measurements.

Supplementary Table 1 ESI source parameters.

	q-TOF	QQQ
Capillary Voltage	-3000 V	-2000 V
Fragmentor	140 V	166 V
Drying Gas Temperature	250°C	150°C
Drying Gas Flow	8 L/min	11 L/min
Nebulizer	8 psi	8 psi

Supplementary Table 2 Optimized fragmentation source parameters, used for CE-QQQ-MS measurements.

Analyte	Precursor Ion	Product Ion	dwel	Fragmentor (V)	Collision Energy (V)	Cell Accelerator Voltage	Polarity
InsP ₁	259	79.1	50	166	41	4	Negative
[¹⁸ O]-1-InsP ₁	263	83	50	166	17	1	Negative
[¹⁸ O]-2-InsP ₁	263	83	50	166	17	1	Negative
[¹⁸ O]-5-InsP ₁	263	83	50	166	17	1	Negative
[¹³ C ₆]-InsP ₁	265	79.1	50	166	41	4	Negative
InsP ₂	339	241	50	166	21	4	Negative
[¹³ C ₆]-InsP ₂	345	247	50	166	21	4	Negative
InsP ₃	418.9	320.8	50	166	17	4	Negative
[¹³ C ₆]-InsP ₃	424.9	326.8	50	166	17	4	Negative
InsP ₄	249	418.9	50	166	5	1	Negative
[¹³ C ₆]-InsP ₄	252	424.9	50	166	5	1	Negative
InsP ₅	289	498.9	50	166	9	3	Negative
[¹³ C ₆]-InsP ₅	292	504.9	50	166	9	3	Negative
InsP ₆	328.9	480.9	50	166	13	4	Negative
[¹³ C ₆]-InsP ₆	331.9	486.9	50	166	13	4	Negative
[¹⁸ O] ₁₂ -InsP ₆	340.9	494.9	50	166	13	4	Negative
InsP ₇	368.9	319.9	50	166	9	3	Negative
[¹³ C ₆]-InsP ₇	371.9	322.9	50	166	9	3	Negative
InsP ₈	408.9	359.8	50	166	9	1	Negative
[¹³ C ₆]-InsP ₈	411.9	362.8	50	166	9	1	Negative

2. Experimental

2.1. Phytase dephosphorylation assay

The phytase assay was performed by incubating the respecting InsP₆ derivatives (15 mM, 0.66 μ L) and 1U enzyme in buffer (HEPES 50 mM, NaCl, 10 mM, glycerol 5%, DTT 2 mM, MgCl₂ 0.5 mM, pH = 4, total reaction volume 15 μ L) at 28°C for 45 min and the reaction outcome was analysed by CE-MS.

Supplementary Table 3 Screened phytases. The activity was expressed as U = μ mol phosphate released per min. All phytases are commercially available and purified prior to use.

Entry	Phytase	Origin	optimal pH	Activity
1	6-phytase	<i>Citrobacter braaki</i>	2.5 to 5.5	30000 U/mL
2	6-phytase	<i>Escherichia coli</i>	3 to 6	7500 U/mL
3	6-phytase	<i>Escherichia coli</i>	2.5 to 6	5000 U/mL
4	3-phytase	<i>Aspergillus niger</i>	4 to 6	10000 U/mL

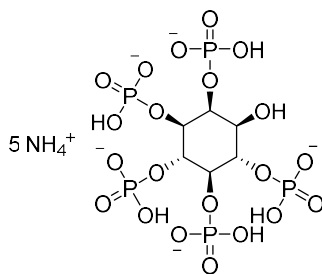
2.1.1. Natuphos dephosphorylations

InsP₆ derivatives were dissolved in NH₄OAc (pH = 6.3), Natuphos (kindly provided by BASF, Art.Nr.: 52587753, Lot.Nr.: 52913677L0, 10500 U/mL at pH = 6.3 with U = μ mol phosphate released per minute) was added and the reaction mixture was incubated at 37°C. The reaction was monitored by ³¹P{¹H}-NMR and the reaction mixture was precipitated in EtOH upon completion.

2.2. Tetrabutylammonium salt formation

To exchange the cations of the here used phosphates, a solution of the respective anion was passed through a column loaded with activated Dowex[®] 50WX8 H⁺ form, a solution of tetrabutylammonium hydroxide (TBA-OH) was added to the eluate and the solution was lyophilized to obtain the resulting TBA salt. The solid residue was equilibrated before and after use with aq. HCl (1 M) and washed with miliQ water until neutral subsequently.

2.3. Synthesis of 1-OH-InsP₅ 2



2

Chemical Formula: C₆H₁₂O₂₁P₅⁵⁻

Molecular Weight: 575.0126

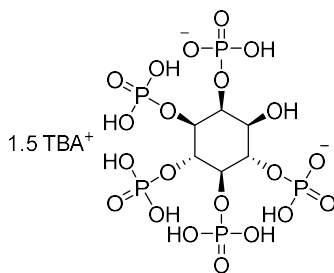
The procedure was adapted from literature.²

InsP₆ (10 g, 8.95 mmol) in NH₄OAc (800 mL, 150 mM, pH = 7) was incubated in the presence of XopH (0.13 μg/mL, 14*10⁻⁶ U/μg with U = μmol phosphate released per minute²) at 28°C for 8 h. The enzyme was deactivated by incubation at 95°C for 15 min and portioned for storage.

A portion (50 mL) was diluted with water (4 × 50 mL), applied onto a Q-sepharose column (XK26) and eluted using a NH₄HCO₃ (1 M, pH = 8) gradient. The fractions were analyzed by ³¹P-NMR and product containing fractions were lyophilized thrice to obtain 1-OH-InsP₅ (255 mg, 383 μmol, 69% for the pentaammonium salt) as a white solid.

Analytical data is in accordance with literature.²

2.4. Salt metathesis of 1-OH-InsP₅ 2



2

Chemical Formula: C₆H₁₂O₂₁P₅⁵⁻

Molecular Weight: 575.0126

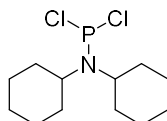
The salt metathesis was performed according to the general procedure (see chapter 2.2).

1-OH-InsP₅ (255 mg, 383 μmol, 1.0 equiv.) was dissolved in water (5 mL), and applied to an activated Dowex[®] 50WX8 H⁺ column. The column was eluted with water until neutral, TBA-OH (20wt% in water, 0.75 mL, 150 mg, 578 μmol, 1.5 equiv.) was added and the solution was lyophilized to obtain a white solid (300 mg, 316 μmol, 82% for 1.5 equiv. TBA⁺).

¹H NMR (400 MHz, Deuterium Oxide) δ = 4.70 (dt, J = 9.2, 2.6 Hz, 1H), 4.41 (q, J = 9.5 Hz, 1H), 4.33 – 4.14 (m, 3H), 3.75 (dt, J = 9.8, 2.2 Hz, 1H), 3.09 – 2.92 (m, 12H), 1.61 – 1.38 (m, 12H), 1.20 (h, J = 7.4 Hz, 12H), 0.78 (t, J = 7.4 Hz, 18H) ppm.

³¹P NMR (162 MHz, Deuterium Oxide) δ = 0.09 (d, J = 9.2 Hz), -0.16 (d, J = 9.3 Hz), -0.61 (d, J = 9.2 Hz), -0.89 (d, J = 9.0 Hz) ppm.

2.5. Synthesis of 1,1-dichloro-*N,N*-dicyclohexylphosphanamine **22**



22

Chemical Formula: C₁₂H₂₂Cl₂NP

Molecular Weight: 282.1888

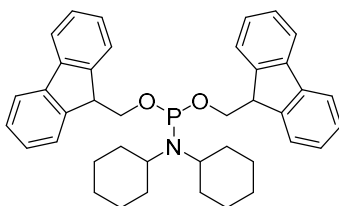
Distilled PCl₃ (5.0 mL, 7.9 g, 58 mmol, 1.0 equiv.) was dissolved in Et₂O (100 mL) in a sulphuration flask and cooled to 0°C. Dicyclohexylamine (23.0 mL, 20.9 g, 115 mmol, 2.0 equiv.) in Et₂O (150 mL) was added dropwise. The reaction mixture was stirred for 2 h and then filtered over neutral Al₂O₃. The solution was concentrated to 80 mL under reduced pressure and incubated at -20°C for 2 days. The formed crystals were filtered, the supernatant was concentrated *in vacuo* and crystalized and the target compound was obtained as colorless crystals (12.0 g, 42.8 mmol, 78%).

¹H NMR (300 MHz, Chloroform-*d*): δ = 3.52-3.35 (m, 2H), 1.94-0.95 (m, 20H) ppm.

¹³C NMR (101 MHz, Chloroform-*d*): δ = 29.44, 26.34, 25.48, 24.96 ppm.

³¹P{¹H} NMR (122 MHz, Chloroform-*d*): δ = 169.65 ppm.

2.6. Synthesis of bis((9*H*-fluoren-9-yl)methyl) dicyclohexylphosphoramidite **1**



1

Chemical Formula: C₄₀H₄₄NO₂P

Molecular Weight: 601.7708

9-Fluorenylmethanol (5.25 g, 16.8 mmol, 1.0 equiv.) was dried in a desiccator, dissolved in THF (150 mL), NEt₃ (5.46 mL, 3.97 g, 39.2 mmol, 2.1 equiv.) was added and the mixture was stirred at 0°C for 30 min. 1,1-dichloro-*N,N*-dicyclohexylphosphanamine **22** (5.25 g, 16.8 mmol, 1.0 equiv.)

was added, the mixture was stirred at 0°C for 30 min and allowed to reach RT overnight. The reaction mixture was filtered over neutral Al₂O₃ and the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography on SiO₂ (cyclohexane:Et₂O, 9:1) to obtain **1** as a colourless sticky solid (9.78 g, 15.5 mmol, 92%).

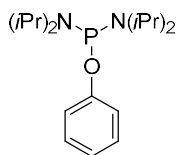
¹H NMR (300 MHz, Chloroform-*d*): δ = 7.73-7.63 (m, 4H), 7.63-7.52 (m, 4H), 7.31 (qd, *J* = 7.5, 1.2 Hz, 4H), 7.26-7.20 (m, 4H), 4.12 (t, *J* = 7.0 Hz, 2H), 3.95 (dt, *J* = 9.8, 6.7 Hz, 2H), 3.75 (dt, *J* = 9.9, 7.2 Hz, 2H), 3.15-2.94 (m, 2H), 1.71-1.47 (m, 10H), 1.37 (ddd, *J* = 15.3, 12.6, 6.5 Hz, 4H), 1.29-1.07 (m, 4 H), 0.97 (qt, *J* = 12.8, 3.3 Hz, 2H) ppm.

¹³C NMR (101 MHz, Chloroform-*d*): δ = 144.97 (d, *J* = 29.6 Hz), 141.37, 127.49, 127.45, 126.94 (d, *J* = 1.9 Hz), 125.61, 125.33, 119.95, 119.88, 68.12, 66.07 (d, *J* = 17.5 Hz), 52.31 (d, *J* = 10.2 Hz), 49.31 (d, *J* = 8.0 Hz), 35.68 (d, *J* = 7.3), 26.81, 25.78, 25.76 ppm

³¹P{¹H} NMR (122 MHz, Chloroform-*d*): δ = 146.55 ppm.

HRMS (ESI) for C₄₀H₄₄O₂NP [M+H]⁺: calculated 602.3182, found 602.3180.

2.7. Synthesis of *N,N,N',N'*-tetraisopropyl-1-phenoxyphosphanediamine **23**



23

Chemical Formula: C₁₈H₃₃N₂OP
Molecular Weight: 324.4488

Phenol (1.68 g, 17.9 mmol, 1.0 equiv.) was dried under high vacuum for 2 h, dissolved in dry Et₂O (40 mL), NEt₃ (2.6 mL, 1.89 g, 18.6 mmol, 1.04 equiv.) was added, the reaction mixture was cooled to -20°C (ice/NaCl) and bis-(diisopropylamino)-chlorophosphine (4.79 g, 17.9 mmol, 1.0 equiv.) was added. The reaction mixture was diluted with Et₂O (50 mL) after 1.5 h and filtered over neutral Al₂O₃, the residue was washed with Et₂O (4 × 100 mL) and the solvent was removed under reduced pressure to obtain colorless crystals (5.43 g, 16.7 mmol, 94%, 97% purity).

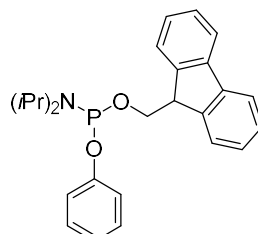
¹H NMR (400 MHz, Chloroform-*d*): δ = 7.22 (dd, *J* = 8.6, 7.4 Hz, 2H), 7.04 (ddt, *J* = 7.8, 2.0, 1.1 Hz, 2H), 6.90 (tt, *J* = 7.3, 1.1 Hz, 1H), 3.61 (dp, *J* = 11.3, 6.8 Hz, 4H), 1.20 (d, *J* = 6.8 Hz, 12H), 1.15 (d, *J* = 6.7 Hz, 12H) ppm.

³¹P NMR (162 MHz, Chloroform-*d*): δ = 120.11 (t, *J* = 11.5 Hz) ppm.

¹³C NMR (101 MHz, Chloroform-*d*): δ = 156.19 (d, *J* = 10.1 Hz), 129.20, 120.72 (d, *J* = 1.5 Hz), 118.88 (d, *J* = 11.8 Hz), 45.01 (d, *J* = 12.5 Hz), 24.41 (d, *J* = 8.9 Hz), 24.05 (d, *J* = 5.4 Hz) ppm.

HRMS (ESI) for $C_{18}H_{33}N_2OP$ $[M+H]^+$: calculated 325.2404, found 325.2406.

2.8. Synthesis of (9H-fluoren-9-yl)methyl phenyl diisopropylphosphoramidite **9**



9

Chemical Formula: $C_{26}H_{30}NO_2P$
Molecular Weight: 419.5048

9-Fluorenylmethanol (1.23 g, 6.27 mmol, 1.0 equiv.) and **23** (2.04 g, 6.27 mmol, 1.0 equiv.) were dried *in vacuo* for 2 h. The reagents were dissolved in dry Et_2O (60 mL) and ETT (100 mg/mL in dry MeCN, 8.2 mL, 820 mg, 6.23 mmol, 1.0 equiv.) was added at 0°C. The reaction mixture was diluted with dry Et_2O (20 mL) after 19 h, filtered over neutral Al_2O_3 , the stationary phase was washed with Et_2O (100 mL) and the solvents were removed under reduced pressure to obtain the crude as a colorless oil (2.26 g, 5.38 mmol, 86%, containing 10% unreacted starting material).

The crude was purified by DCVC on SiO_2 using a cyclohexan:EtOAc solvent mixture containing NEt_3 (5 %) and solvents were removed *in vacuo* to obtain a colorless oil (587 mg, 1.40 mmol, 22%).

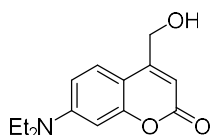
1H NMR (400 MHz, Chloroform-*d*): δ = 7.77 (dq, J = 7.6, 1.1 Hz, 2H), 7.69 (dq, J = 7.5, 0.9 Hz, 1H), 7.61 (dq, J = 7.5, 0.9 Hz, 1H), 7.40 (tddd, J = 7.5, 3.5, 1.2, 0.7 Hz, 2H), 7.34 – 7.23 (m, 4H), 7.10 – 7.04 (m, 2H), 7.04 – 6.99 (m, 1H), 4.28 – 4.22 (m, 1H), 4.11 (dt, J = 9.9, 6.7 Hz, 1H), 3.92 (dt, J = 9.9, 7.4 Hz, 1H), 3.78 (dp, J = 10.5, 6.8 Hz, 2H), 1.21 (d, J = 3.6 Hz, 6H), 1.20 (d, J = 3.6 Hz, 6H) ppm.

^{13}C NMR (101 MHz, Chloroform-*d*): δ = 154.86 (d, J = 7.4 Hz), 144.95, 144.58, 141.47 (d, J = 7.9 Hz), 129.48, 127.61, 127.58, 127.04, 126.99, 125.60, 125.39, 122.39 (d, J = 1.6 Hz), 120.31, 120.22, 119.98, 119.95, 66.61 (d, J = 17.2 Hz), 49.20 (d, J = 7.9 Hz), 43.73, 43.60, 24.78, 24.71, 24.63, 24.55 ppm.

$^{31}P\{^1H\}$ -NMR (162 MHz, Chloroform-*d*): δ = 145.79 ppm.

HRMS (ESI) for $C_{26}H_{30}O_2NP$ $[M+H]^+$: calculated 420.2087, found 420.2084.

2.9. Synthesis of (diethylamino)-4-(hydroxymethyl)-2H-chromen-2-one 24



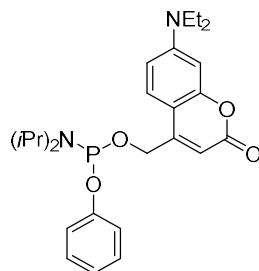
24

Chemical Formula: C₁₄H₁₇NO₃

Molecular Weight: 247.2940

20 was synthesized according to literature.³

2.10. Synthesis of (7-(diethylamino)-2-oxo-2H-chromen-4-yl)methyl phenyl diisopropylphosphoramidite 10



10

Chemical Formula: C₂₆H₃₅N₂O₄P

Molecular Weight: 470.5498

19 (349 mg, 1.08 mmol, 1.0 equiv.) and DEACM-OH **24** (267 mg, 1.08 mmol, 1.0 equiv.) were dried *in vacuo* for 4.5 h. The reagents were dissolved in dry Et₂O:CH₂Cl₂ (1:1, 30 mL) and ETT (100 mg/mL in dry MeCN, 1.4 mL, 1.08 mmol, 1.0 equiv.) was added at 0°C. The reaction mixture was diluted with Et₂O (50 mL) after 17.5 h, filtered over neutral Al₂O₃, the stationary phase was washed with Et₂O (100 mL) and the solvents were removed under reduced pressure to obtain a yellow oil (440 mg, 935 μmol, 87%).

¹H NMR (400 MHz, Chloroform-*d*): δ = 7.33 – 7.22 (m, 3H), 7.10 – 6.98 (m, 3H), 6.58 – 6.49 (m, 2H), 6.28 (t, *J* = 1.4 Hz, 1H), 4.86 (ddd, *J* = 7.6, 6.7, 1.4 Hz, 2H), 3.79 (dp, *J* = 10.7, 6.8 Hz, 2H), 3.40 (q, *J* = 7.1 Hz, 4H), 1.26 (d, *J* = 6.8 Hz, 6H), 1.20 (t, *J* = 7.1 Hz, 6H), 1.20 (d, *J* = 6.9 Hz, 6H).

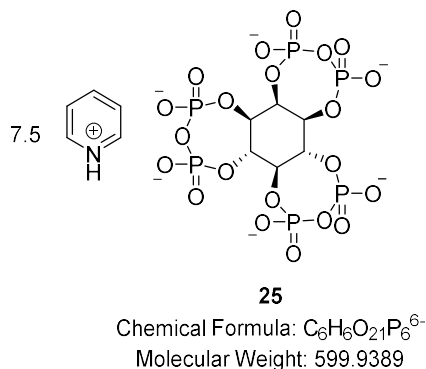
¹³C NMR (101 MHz, Chloroform-*d*) δ 162.41, 156.30, 154.54 (d, *J* = 7.2 Hz), 152.58 (d, *J* = 7.6 Hz), 150.58, 129.59, 124.53, 122.70 (d, *J* = 1.7 Hz), 120.17 (d, *J* = 8.9 Hz), 108.57, 106.55, 97.96, 62.05 (d, *J* = 18.7 Hz), 44.87, 43.93 (d, *J* = 12.9 Hz), 24.91 (d, *J* = 7.1 Hz), 24.53 (d, *J* = 7.2 Hz), 12.59 ppm.

³¹P{¹H} NMR (162 MHz, Chloroform-*d*): δ = 147.30 ppm.

^{13}C NMR (101 MHz, Chloroform-*d*): δ = 162.41, 156.30, 154.54 (d, J = 7.2 Hz), 152.58 (d, J = 7.6 Hz), 150.58, 129.59, 124.53, 122.70 (d, J = 1.7 Hz), 120.17 (d, J = 8.9 Hz), 108.57, 106.55, 97.96, 62.05 (d, J = 18.7 Hz), 44.87, 43.93 (d, J = 12.9 Hz), 24.91 (d, J = 7.1 Hz), 24.53 (d, J = 7.2 Hz), 12.59 ppm.

HRMS (ESI) for $\text{C}_{26}\text{H}_{35}\text{O}_4\text{N}_2\text{P}$ $[\text{M}+\text{Na}]^+$: calculated 493.2227, found 493. 2232.

2.11. Synthesis of Inositoltrispyrophosphate 25



Based on literature procedures^{4,5}:

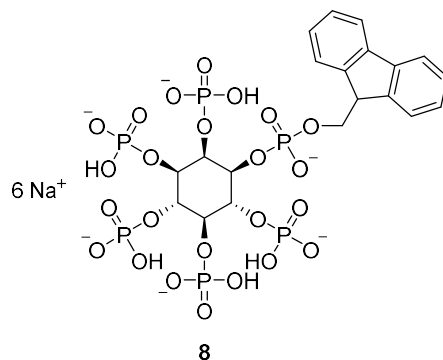
Myo-inositolhexakisphosphate dodecasodium salt (1.07 g, 0.96 mmol, 1.0 equiv.) was passed through an activated DOWEX H^+ column, the eluate was adjusted to pH = 8 with pyridine and the solution was lyophilised. The obtained *myo*-inositolhexakisphosphate pyridinium salt was dissolved in water (10 mL) and added to a solution of dicyclohexylcarbodiimide (1.8 g, 8.72 mmol, 9.1 equiv.) in pyridine (30 mL) and heated to 70°C for 8 h. After stirring at RT overnight dicyclohexylcarbodiimide (750 mg, 3.63 mmol, 3.8 equiv.) was added and the reaction mixture was heated to 70°C for 10 h. After stirring at RT overnight dicyclohexylcarbodiimide (1.4 g, 6.79 mmol, 7.1 equiv.) was added and the reaction mixture was heated to 70°C for 8 h. The solvents were evaporated under reduced pressure, the resulting solid was suspended in water (20 mL) and filtered, the white residue was washed with water (4×20 mL) and the filtrate was lyophilized to obtain a white hygroscopic solid (988 mg, 0.82 mmol, 86%).

^1H NMR (400 MHz, Deuterium Oxide): δ = 8.87 – 8.76 (m, 15H), 8.65 (tt, J = 7.8, 1.4 Hz, 8H), 8.10 (t, J = 6.9 Hz, 15H), 5.21 – 5.11 (m, 1H), 4.70 (dt, J = 9.0, 4.3 Hz, 1H), 4.56 – 4.49 (m, 2H), 4.44 – 4.31 (m, 2H) ppm.

$^{31}\text{P}\{^1\text{H}\}$ NMR (162 MHz, Deuterium Oxide): δ = -9.69 (d, J = 22.1 Hz), -10.75 – -11.14 (m), -11.26, -14.49 (d, J = 21.9 Hz) ppm.

HRMS (ESI) for $\text{C}_6\text{H}_{12}\text{O}_{21}\text{P}_6$ $[\text{M}-2\text{H}]^{2-}$: calculated 301.9075, found 301.9082.

2.12. Synthesis of 1-Fm-InsP₆ **8**



Chemical Formula: C₂₀H₂₂O₂₄P₆⁶⁻

Molecular Weight: 832.2179

1-OH-InsP₅ × 1.15 tetrabutylammonium salt **2** (165 mg, 172 μmol, 1.0 equiv.) and **9** (579 mg, 1.38 mmol, 8.0 equiv.) were dissolved in dry MeCN (5.0 mL) and co-evaporated. ETT (300 mg, 2.31 mmol, 13.4 equiv.) was dissolved in dry MeCN (5.0 mL) and co-evaporated. The reagents were dissolved in dry DMF (16.0 mL), ETT was dissolved in dry MeCN (3.0 mL) and added to the reaction mixture at RT. Pyridinium Hydrobromide (244 mg, 1.53 mmol, 8.9 equiv.) was added after 22 h, *m*CPBA (70%wet, 235 mg, 953 μmol, 5.5 equiv.) was added at 0°C after 15 min and the reaction mixture was precipitated in ice cold Et₂O (8 vol.) in portions after 30 min. The solid residue was washed with acetone (30 mL) and dried under ambient conditions to obtain an off-white solid. The crude was dissolved in D₂O (5 mL), HBr (33% in acetic acid, 0.7 mL) was added and the reaction was heated at 80°C. The reaction mixture was diluted with water (30 mL) after 7 h, NH₄HCO₃ buffer (1 M, pH = 8, 5 mL) was added and the crude was purified by SAX on a Q-sepharose column (*XK16/20*) on a NaClO₄ (1 M) gradient. Product containing fractions were lyophilised, the residue was washed with acetone (2 × 20 mL), dissolved in water and lyophilised to obtain a white solid (75 mg, 77 μmol, 45% for the hexasodium salt).

NMR for NH₄⁺ cations

¹H NMR (400 MHz, Deuterium Oxide): δ = 7.98 – 7.86 (m, 4H), 7.60 – 7.46 (m, 4H), 5.01 (d, *J* = 9.7 Hz, 1H), 4.58 – 4.35 (m, 5H), 4.23 (q, *J* = 9.5 Hz, 1H), 4.15 (t, *J* = 9.5 Hz, 1H), 4.05 (t, *J* = 9.4 Hz, 1H) ppm.

³¹P NMR (162 MHz, Deuterium Oxide): δ = 1.75 (d, *J* = 9.9 Hz), 1.01 (d, *J* = 9.9 Hz), 0.36 (d, *J* = 9.6 Hz), -0.51 (d, *J* = 9.3 Hz), -0.75 (dd, *J* = 6.3 Hz) ppm.

^{13}C NMR (101 MHz, Deuterium Oxide): δ : = 144.35, 144.23, 140.96, 140.91, 127.95, 127.91, 127.56, 127.53, 125.81, 125.60, 120.04, 120.01, 77.30, 76.26, 75.17 (d, J = 7.5 Hz), 75.04 (d, J = 3.4 Hz), 73.36, 73.17, 67.54 (d, J = 5.9 Hz), 48.05 (d, J = 8.0 Hz) ppm.

NMR for Na^+ cations

^1H NMR (400 MHz, Deuterium Oxide): δ = 7.99 – 7.85 (m, 4H), 7.59 – 7.45 (m, 4H), 5.01 (t, J = 2.4 Hz, 1H), 4.55 (t, J = 9.4 Hz, 1H), 4.51 (t, J = 9.4 Hz, 1H), 4.58 – 4.38 (m, 3H), 4.25 (t, J = 9.4 Hz, 1H), 4.17 (dd, J = 9.8, 2.5 Hz, 1H), 4.07 (dd, J = 10.0, 2.3 Hz, 1H) ppm.

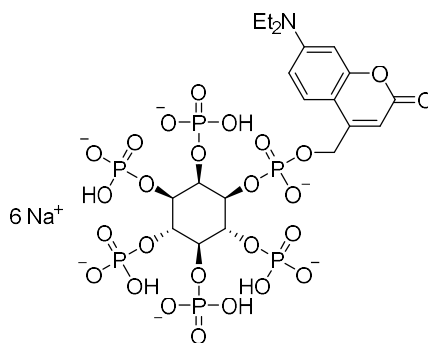
^{31}P NMR (162 MHz, Deuterium Oxide): δ = 1.80 (d, J = 9.7 Hz), 1.09 (d, J = 9.7 Hz), 0.42 (d, J = 9.5 Hz), 0.39 (d, J = 9.2 Hz), -0.37 – -0.76 (m) ppm.

^{13}C NMR (101 MHz, Deuterium Oxide): δ = 144.36, 144.26, 140.95, 140.89, 127.94, 127.89, 127.57, 127.53, 125.85, 125.63, 120.02, 119.99, 77.37, 76.19 (td, J = 6.2, 2.6 Hz), 75.21 (d, J = 6.5 Hz), 75.05 (dq, J = 6.4, 3.0 Hz), 73.46 (dd, J = 5.8, 2.8 Hz), 73.20 (dd, J = 6.1, 3.2 Hz), 67.55 (d, J = 5.7 Hz), 48.05 (d, J = 8.1 Hz) ppm.

HRMS (ESI) for $\text{C}_{20}\text{H}_{28}\text{O}_{24}\text{P}_6$ $[\text{M}-\text{H}]^-$: calculated 836.9323, found 836.9328.

$[\alpha]_D^{20}$ = 3.6 (0.25, water).

2.13. Synthesis of 1-DEACM-InsP₆ **11**



11

Chemical Formula: $\text{C}_{20}\text{H}_{27}\text{NO}_{26}\text{P}_6^{6-}$
Molecular Weight: 883.2629

1-OH-InsP₅ $\times$ 1.57 tetrabutylammonium salt **2** (23 mg, 24 μmol , 1.0 equiv.) was co-evaporated with dry MeCN (2×3 mL), the reagent was dissolved in dry DMF (1.0 mL) and **10** (88 mg, 187 μmol , 7.8 equiv.) and ETT (100 mg/mL in dry MeCN, 0.4 mL, 40 mg, 307 μmol , 12.8 equiv.) were added. Pyridinium hydrobromide (32 mg, 200 μmol , 8.3 equiv.) was added after 16 h, *m*CPBA (70%wet, 62 mg, 252 μmol , 10.5 equiv.) was added at 0°C after 45 min, the reaction mixture was precipitated in ice cold Et₂O (40 mL) after 30 min. The residue was dissolved in D₂O (1 mL), ZnCl₂ (114 mg, 837 μmol , 34.9 equiv.) and HCl_{conc.} (0.35 mL, 154 mg, 4.24 mmol, 177 equiv.) were

added and the reaction mixture was incubated at 37°C for 19 h. The solution was diluted with water (20 mL) and NH_4HCO_3 buffer (1 M, 5 mL, pH = 8) and purified by SAX on a Q-sepharose column (*XK16/20*) on a NaClO_4 (1 M) gradient. Product containing fractions were lyophilised, washed with acetone (2×5 mL) and dried *in vacuo* to obtain a yellow solid (8 mg, 8 μmol , 35% for the hexasodium salt).

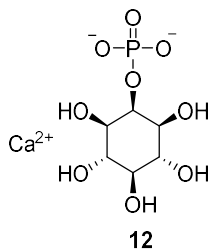
^1H NMR (400 MHz, D_2O): δ = 7.75 (d, J = 9.1 Hz, 1H), 6.92 (dd, J = 9.1, 2.4 Hz, 1H), 6.72 (d, J = 2.5 Hz, 1H), 6.37 (d, J = 1.4 Hz, 1H), 5.40 (d, J = 8.5 Hz, 2H), 5.08 (d, J = 9.5 Hz, 1H), 4.61 – 4.43 (m, 3H), 4.30 – 4.11 (m, 4H), 3.50 (q, J = 7.1 Hz, 6H), 1.22 (t, J = 7.1 Hz, 9H) ppm.

^{13}C NMR (101 MHz, D_2O): δ = 166.03, 155.66, 155.60, 155.55, 151.27, 125.88, 110.31, 106.18, 103.54, 97.34, 77.41, 74.51, 73.61, 63.84 (d, J = 5.0 Hz), 44.48, 11.52 ppm.

^{31}P NMR (162 MHz, D_2O): δ = 2.79 (d, J = 10.0 Hz), 1.85, 1.18 (d, J = 9.7 Hz), 0.77 (d, J = 9.9 Hz), -0.20 (dt, J = 9.1, 9.1 Hz) ppm.

HRMS (ESI) for $\text{C}_{20}\text{H}_{33}\text{NO}_{26}\text{P}_6$ $[\text{M}-2\text{H}]^{2-}$: calculated 443.4785, found 443.4789.

2.14. Synthesis of 2-InsP₁ 12



12

Chemical Formula: $\text{C}_6\text{H}_{11}\text{CaO}_9\text{P}$
Molecular Weight: 298.1968

InsP₆-dodecasodium salt (1.00 g, 901 μmol) was dissolved in NH_4OAc buffer (50 mM, pH = 6.3, 6.0 mL) Natuphos (300 μL , 10500 U/mL) was added and the solution was incubated at 37°C for 10 min. The reaction mixture was precipitated in EtOH (4 vol.), the precipitate was dissolved in water (20 mL) and precipitated by addition of CaCl_2 (640 mg, 5.77 mmol, 6.4 equiv.). The motherliquor was precipitated by addition of acetone (4 vol.) in two portions to obtain a white solid. The CaCl_2 -precipitation of inorganic calcium phosphate and the following acetone precipitation of 2-InsP₁ was repeated six times, the resulting precipitate was dried under reduced pressure to obtain a white solid (231 mg, 775 μmol , 86% for the calcium salt).

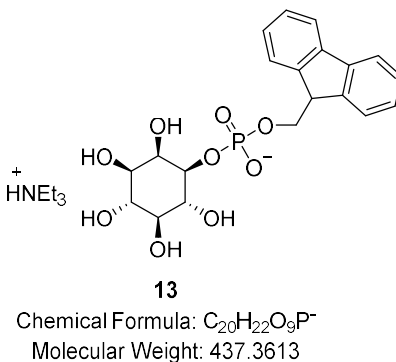
^1H NMR (400 MHz, D_2O) δ = 4.56 (dt, J = 7.5, 2.5 Hz, 1H), 3.73 (t, J = 9.6 Hz, 2H), 3.54 (ddd, J = 9.9, 2.5, 1.2 Hz, 2H), 3.29 (t, J = 9.3 Hz, 1H) ppm.

^{13}C NMR (101 MHz, D_2O) δ = 76.13 (d, J = 5.6 Hz), 74.42, 72.88, 71.32, 71.29 ppm.

³¹P NMR (162 MHz, D₂O) δ = 3.02 (d, *J* = 7.5 Hz) ppm.

HRMS (ESI) for C₆H₁₃O₉P [M-H]⁻: calculated 259.0224, found 259.0231.

2.15. Synthesis of 1-Fm-InsP₁ 13



1-Fm-InsP₆ **8** (50 mg, 52 μmol) was dissolved in NH₄OAc (50 mM in D₂O, pH = 6.3, 0.8 mL), Natuphos (0.2 mL, 10500 U/mL) was added and the reaction as incubated at 37°C for 2 days. The reaction mixture was precipitated in EtOH (30 mL), the precipitate was dissolved in water (1 mL), purified by MPLC on a C₁₈-Aq column using a Water:MeCN gradient (containing 10% triethylammonium acetate buffer and product containing fractions were lyophilised twice to obtain a white solid (3 mg). The motherliquor was concentrated *in vacuo*, the residue was dissolved in water (1 mL), purified by MPLC and product containing fractions were lyophilised twice to obtain a white solid. The material was used directly for subsequent basic deprotection.

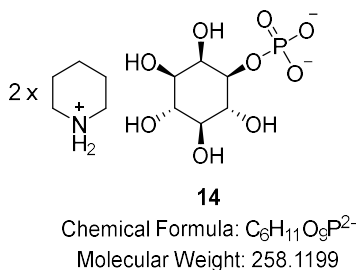
¹H NMR (400 MHz, Deuterium Oxide): δ = 7.96 (dt, J = 8.0, 0.9 Hz, 2H), 7.84 – 7.76 (m, 2H), 7.55 (td, J = 7.5, 1.3 Hz, 2H), 7.51 – 7.44 (m, 2H), 4.50 – 4.42 (m, 1H), 4.41 – 4.31 (m, 2H), 3.91 (t, J = 2.8 Hz, 1H), 3.71 – 3.52 (m, 2H), 3.32 (dd, J = 10.0, 2.9 Hz, 1H), 3.23 (q, J = 7.3 Hz, 6H, HNEt₃⁺), 3.18 (t, J = 9.2 Hz, 1H), 1.31 (t, J = 7.3 Hz, 9H, HNEt₃⁺) ppm.

¹³C NMR (101 MHz, Deuterium Oxide): δ = 144.23, 144.11, 141.03, 140.99, 128.02, 127.48, 125.32, 125.16, 120.16 (d, *J* = 2.5 Hz), 76.04 (d, *J* = 6.1 Hz), 73.83, 72.19, 71.29, 70.95, 70.62, 66.93 (d, *J* = 5.6 Hz), 46.69, 8.22 ppm.

³¹P{¹H} NMR (162 MHz, Deuterium Oxide): δ = -0.34 ppm.

HRMS (ESI) for C₂₀H₂₃O₉P [M-H]⁻: calculated 437.1006, found 437.1008.

2.16. Synthesis of 1-InsP₁ **14**



1-Fm-InsP₁ **13** (Starting from 1-Fm-InsP₆ **8** 50 mg, 52 μmol) was dissolved in water (5 mL), piperidine (0.5 mL) was added and the reaction mixture was incubated at RT for 30 min. The reaction mixture was precipitated in acetone (8 vol.), washed with acetone (10 mL) and the precipitate was discarded. The motherliquor was concentrated *in vacuo* to obtain a brown solid. (13 mg, 30 μmol, 58% starting from **158**; containing 2 equiv. piperidinium ions based on NMR.)

¹H NMR (400 MHz, D₂O): δ = 4.15 (t, *J* = 2.8 Hz, 1H), 3.83 (ddd, *J* = 9.8, 8.5, 2.8 Hz, 1H), 3.68 (t, *J* = 9.6 Hz, 1H), 3.57 (dd, *J* = 10.0, 9.1 Hz, 1H), 3.50 (dd, *J* = 10.0, 2.9 Hz, 1H), 3.26 (t, *J* = 9.2 Hz, 1H), 3.13 – 3.02 (m, 8H), 1.76 – 1.65 (m, 8H), 1.63 – 1.53 (m, 4H) ppm.

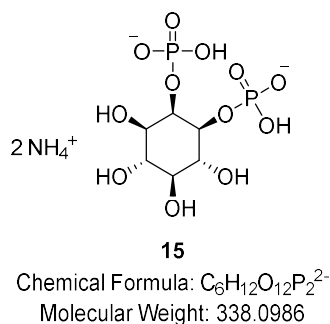
¹³C NMR (101 MHz, D₂O): δ = 74.42, 74.31 (d, *J* = 5.1 Hz), 72.33, 72.25 (d, *J* = 3.8 Hz), 71.71 (d, *J* = 3.0 Hz), 70.85, 44.52, 22.21, 21.49 ppm.

³¹P NMR (162 MHz, D₂O): δ = 3.96 (d, *J* = 8.4 Hz) ppm.

HRMS (ESI) for C₆H₁₃O₉P [M-H]⁻: calculated 259.0224, found 259.0230.

[α]_D²⁰ = 3.1 (0.45, water, pH = 9).

2.17. Synthesis of 1,2-InsP₂ **15**



1-Fm-InsP₆ (15 mM, 0.66 μL) was digested by 6-Phytase from *Escherichia coli* (7500 U/mL, 1 U) in buffer (HEPES 50 mM, NaCl, 10 mM, glycerol 5%, DTT 2 mM, MgCl₂ 0.5 mM, pH = 4, total reaction volume 15 μL) at 28°C for 45 min and the solution was purified by MPLC on a C₁₈-Aq column using a Water:MeCN gradient (containing 10% triethylammonium acetate buffer and

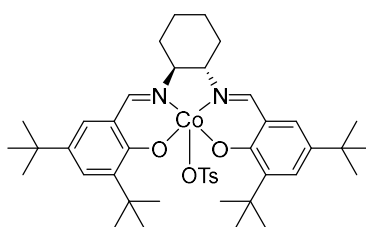
product containing fractions were lyophilised twice to obtain a white solid (**HRMS** (CE-ESI-MS) for $C_{20}H_{23}O_{12}P_2$ $[M-H]^-$: calculated 517.0670, found 517.0671.).

The solid was dissolved in water (0.5 mL), piperidine (50 μ L) was added and the resulting suspension was filtered after 30 min. The solution was lyophilized to obtain a brown solid. The crude was purified by SAX on a Q-sepharose column (*Capto Q-ImpRes*, 1 mL) on a NH_4HCO_3 gradient and product containing fractions were lyophilized to obtain a colorless solid (0.4 mg, quant.).

HRMS (CE-ESI-MS) for $C_6H_{14}O_{12}P_2$ $[M-H]^-$: calculated 338.9888, found 338.9892.

^{31}P NMR (162 MHz, Deuterium Oxide) δ = 4.85, 4.55 ppm.

2.18. Synthesis of Co(III) catalyst 19



19

Chemical Formula: $C_{43}H_{59}CoN_2O_5S$
Molecular Weight: 774.9472

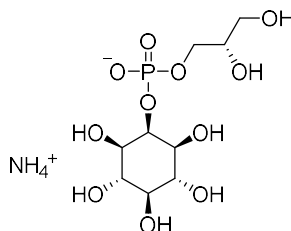
*Based on literature*⁶:

S,S-Co(II)-Salen (105 mg, 0.174 mmol, 1.0 equiv.) and *p*TsOH (36 mg, 0.189 mmol, 1.09 equiv.) were dissolved in CH_2Cl_2 (3 mL) and stirred with an open flask for 1.15 h. The reaction mixture was precipitated in ice cold pentane (24 mL), the motherliquor was concentrated *in vacuo*, the precipitates were combined, washed with pentane (25 mL) and dried under reduced pressure to obtain a green solid.

*Analytical data is in accordance with literature*⁶:

1H NMR (300 MHz, $DMSO-d_6$): δ = 7.78 (s, 2H), 7.52 – 7.40 (m, 6H), 7.15 – 7.06 (m, 2H), 3.66 – 3.56 (m, 2H), 3.11 – 3.01 (m, 2H), 2.28 (s, 3H), 2.05 – 1.87 (m, 2H), 1.74 (s, 18H), 1.66 – 1.64 (m, 2H), 1.67 – 1.54 (m, 2H), 1.30 (s, 18H) ppm.

2.19. Synthesis of 2-glycerophosphoinositol **20**



20

Chemical Formula: $C_9H_{18}O_{11}P^-$

Molecular Weight: 333.2063

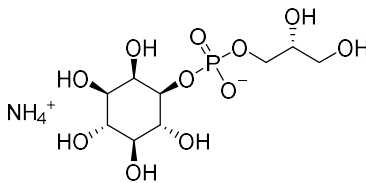
A stock solution of **19** (1 mg/mL, 99 μ L, 767 μ g, 1 μ mol, 10 mol%) in MeCN was added to a solution of 2-InsP₁ $\times$ 2.0 TBA **12** (8.5 mg, 10 μ mol, 1.0 equiv., 0.5 equiv. of TBA-OH were present as minor impurity) in MeCN (1.0 mL), DIPEA (2 μ L; 1.48 mg, 12 μ mol, 1.2 equiv.) and *S*-(-)-glycidol (2 μ L, 2.23 mg, 30 μ mol, 3.1 equiv.) was added and the reaction mixture was stirred with an open flask for 5 min, closed to the atmosphere with a septum and stirred overnight. The reaction mixture was diluted with water (8 mL) and purified by SAX on a Q-sepharose column (*Capto Q-ImpRes*, 1 mL) on a NH_4HCO_3 gradient. Fractions were analyzed by CE-qTOF-MS and product containing fractions were lyophilized thrice to obtain a white solid (1 mg, 3 μ mol, 29% for the ammonium salt).

¹H NMR (400 MHz, Deuterium Oxide): δ = 4.61 (dt, J = 7.8, 2.4 Hz, 2H), 4.09 – 4.00 (m, 2H), 4.02 – 3.91 (m, 3H), 3.76 – 3.67 (m, 4H), 3.68 – 3.56 (m, 5H), 3.32 (t, J = 9.3 Hz, 2H) ppm.

³¹P NMR (162 MHz, Deuterium Oxide): δ = 1.06 (ddd, J = 6.6 Hz) ppm.

HRMS (ESI) for $C_9H_{19}O_{11}P$ [M-H]⁻: calculated 333.0592, found 333.0568.

2.20. Synthesis of 1-glycerophosphoinositol **21**



21

Chemical Formula: $C_9H_{18}O_{11}P^-$

Molecular Weight: 333.2063

A stock solution of **19** in MeCN (10 mM, 123 μ L, 10 mol%) was added to 1-InsP₁ $\times$ 2.0 TBA **14** (10.0 mg, 12 μ mol, 1.0 equiv., 0.3 equiv. of TBA-OH were present as minor impurity) in MeCN (1.0 mL), DIPEA (2.5 μ L, 14 μ mol, 1.2 equiv.) and *S*-(-)-glycidol (2.5 μ L, 28 μ g, 3.1 equiv.) were added and the reaction mixture was stirred with an open flask for 5 min, closed to the atmosphere

with an septum and stirred overnight. The reaction mixture was diluted with water (8 mL) and purified by SAX on a Q-sepharose column (*Capto Q-ImpRes*, 1 mL) on a NH_4HCO_3 gradient. Fractions were analyzed by CE-qTOF-MS and product containing fractions were lyophilized thrice to obtain a white solid (1 mg, 3 μmol , 25% for the ammonium salt).

Analytical data is in accordance with literature⁷:

$^1\text{H}\{^{31}\text{P}\}$ NMR (400 MHz, Deuterium Oxide): δ = 4.29 (dd, J = 2.8 Hz, 1H), 4.00 (dd, J = 9.9, 2.8 Hz, 1H), 4.04 – 3.90 (m, 3H), 3.78 (dd, J = 9.7 Hz, 1H), 3.74 – 3.61 (m, 3H), 3.58 (dd, J = 10.0, 2.8 Hz, 1H), 3.36 (dd, J = 9.4 Hz, 1H) ppm.

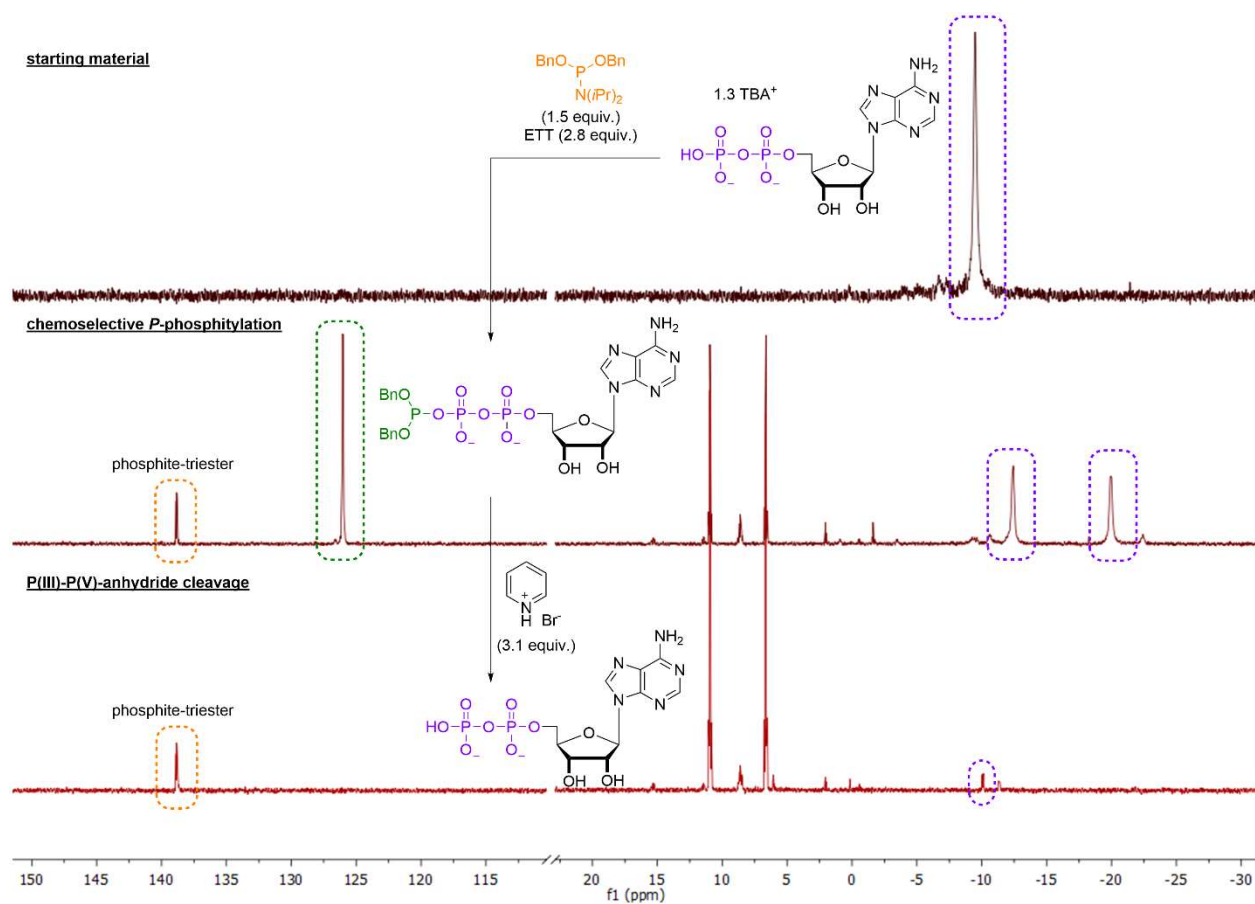
^{13}C NMR (101 MHz, Deuterium Oxide): δ = 76.29, 73.94, 72.22, 71.36 (d, J = 6.8 Hz), 71.18, 70.79, 70.74, 66.40 (d, J = 5.9 Hz), 62.07 ppm.

$^{31}\text{P}\{^1\text{H}\}$ NMR (162 MHz, Deuterium Oxide): δ = -0.05 ppm.

HRMS (ESI) for $\text{C}_9\text{H}_{19}\text{O}_{11}\text{P}$ $[\text{M}-\text{H}]^-$: calculated 333.0592, found 333.0588.

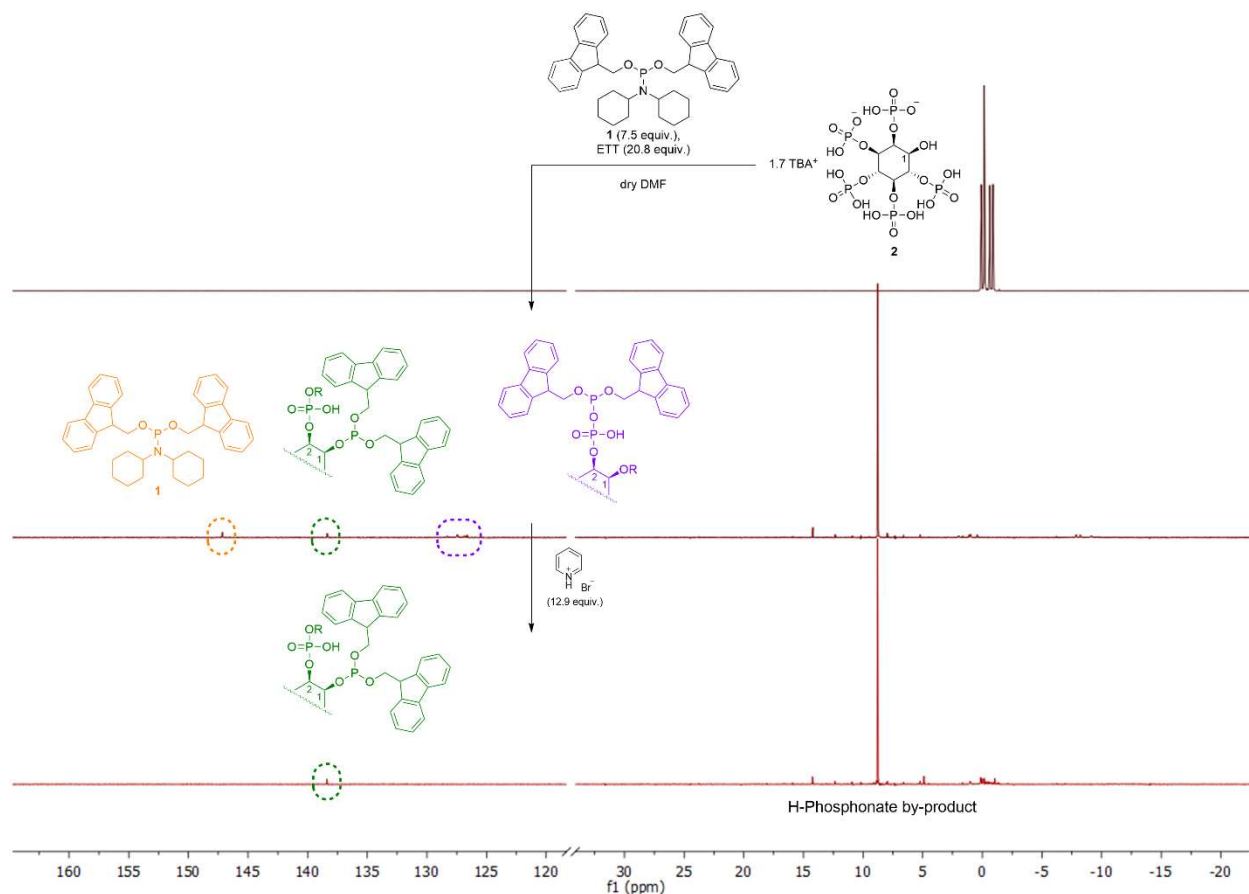
3. Transient Phosphitylation monitored by ^{31}P -NMR

3.1. Transient Phosphitylation of ADP



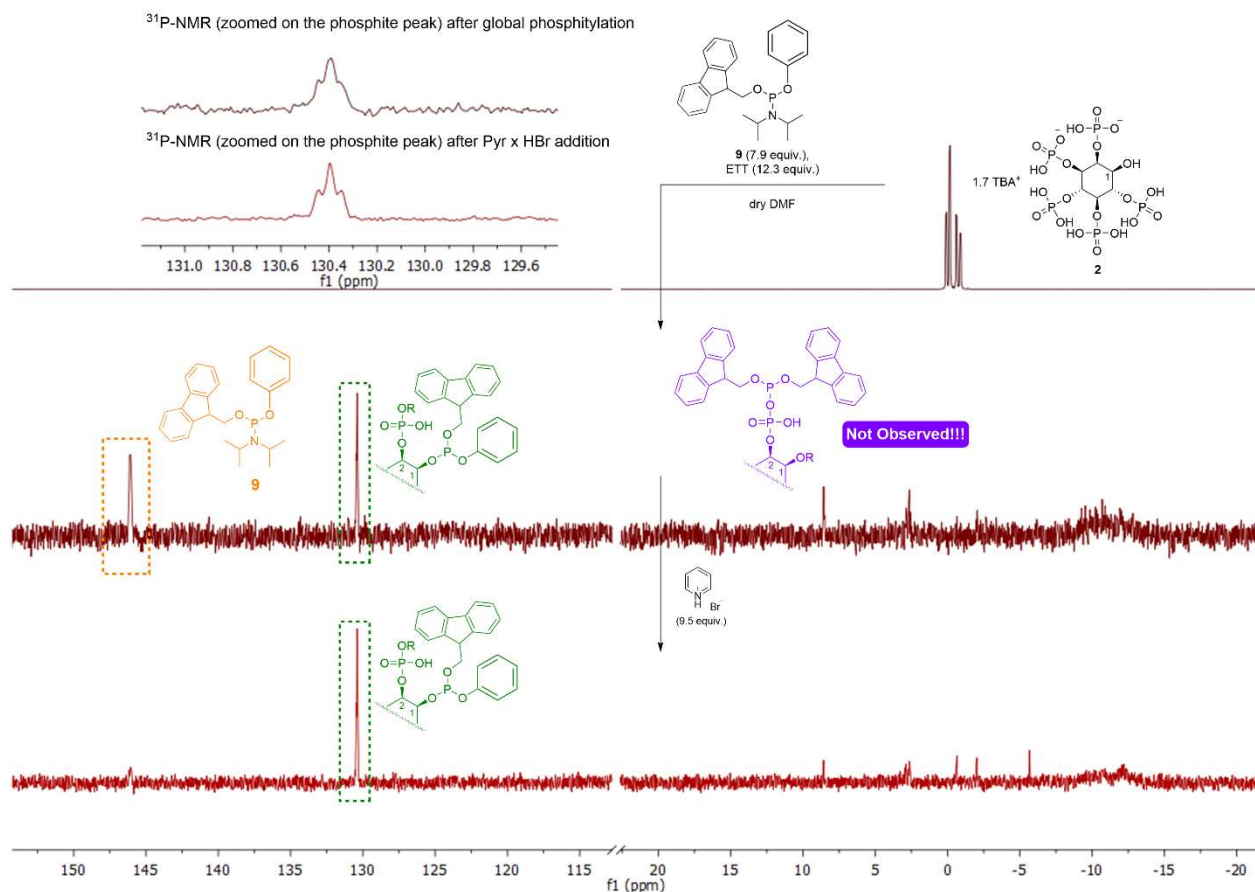
Supplementary Figure 1 Transient Phosphitylation of ADP monitored via ^{31}P -NMR. Coupling of ADP with commercially available dibenzyl-*N,N*-diisopropyl-P-amidite led to a P(III)-P(V) anhydride. Subsequent addition of pyridinium hydrobromide led to cleavage of the P(III)-P(V) anhydride. The chemical shifts of the observed intermediates are in accordance with literature.⁸

3.2. Transient Phosphitylation of 1-OH-InsP₅ with P-amidite 1



Supplementary Figure 2 Transient Phosphitylation of 1-OH-InsP₅ **2** monitored via $^{31}\text{P}\{^1\text{H}\}$ -NMR. Coupling of **2** with P-amidite **1** led to a complex mixture of P(III)-P(V) anhydrides and to a P(OR)₃-species. Subsequent addition of pyridinium hydrobromide led to cleavage of the P(III)-P(V) anhydrides, while the P(OR)₃-species stayed intact. The chemical shifts of the observed intermediates are in accordance with literature.⁸

3.3. Transient Phosphitylation of 1-OH-InsP₅ with P-amidite 9



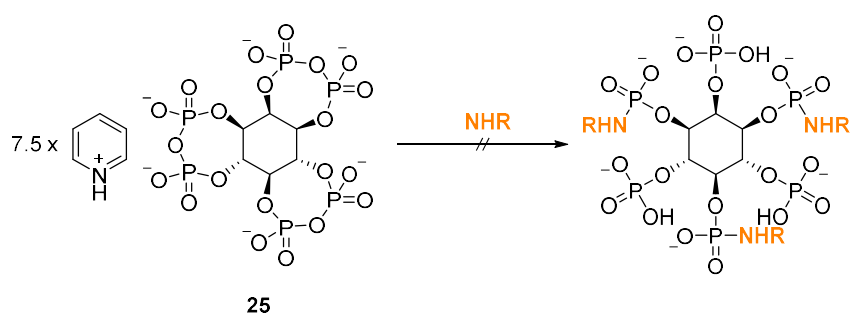
Supplementary Figure 3 Transient Phosphitylation of 1-OH-InsP₅ **2** monitored *via* ³¹P-NMR. Coupling of **2** with P-amidite **9** led to a P(OR)₃-species, while no P(III)-P(V)-anhydrides were detectable. Subsequent addition of pyridinium hydrobromide led to complete consumption of unreacted P-amidite, P(OR)₃-species stayed intact. The chemical shifts of the observed intermediates are in accordance with literature.⁸

4. Towards the linearization of cyclic inositolpyrophosphates to InsP₆ derivatives

4.1. Screening for conditions to ring open the cyclic pyrophosphates of inositoltrispyrophosphate (ITPP)

4.1.1. Attempted nucleophilic ring-opening of ITPP with amines

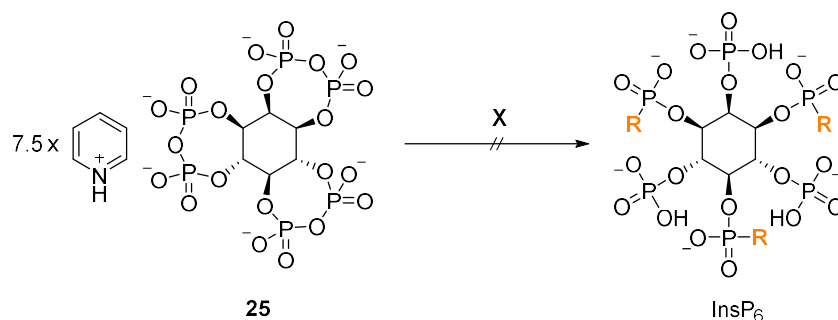
Supplementary Table 4 Ring-opening screening of ITPP **25** with amine nucleophiles at RT. If not otherwise stated, 20 vol% amine were used. ¹Based on CE-qTOF-MS analysis (BGE: NH₄OAc 35 mM pH 9.7, CE voltage 30 kV, CE current: 23 μA, injection 100 mbar, 15 s (30 nL)) and ³¹P-NMR. ²Based on CE-qTOF-MS analysis (BGE: NH₄OAc 35 mM pH = 9.7, CE voltage 30 kV, CE current: 23 μA, injection 100 mbar, 15 s (30 nL)) ring opening occurred to some degree, but was not quantifiable by NMR.



Entry	Amine	Reaction Time	Solvent	Outcome ¹
1	Dimethyl amine (40 vol%)	Overnight	Water	/ [*]
2	<i>o</i> -Hydroxylaniline (128 equiv.)	Overnight	Water:DMSO (5:2)	/
3	Hydroxylamine Hydrochloride (103 equiv.)	Overnight	Water	/
4	Morpholine	Overnight	Water	/ ²
5	Morpholine	Overnight	MeCN	/
6	Hydrazine	3 days	Water	One opened pyrophosphate
7	Hydrazine	Overnight	MeCN	/
8	Propargyl Amine	3 days	Water	/ ²
9	Ammonia	3 days	Water	/ ²
10	Piperidine	Overnight	MeCN	/
11	Piperidine	Overnight	DMF	/

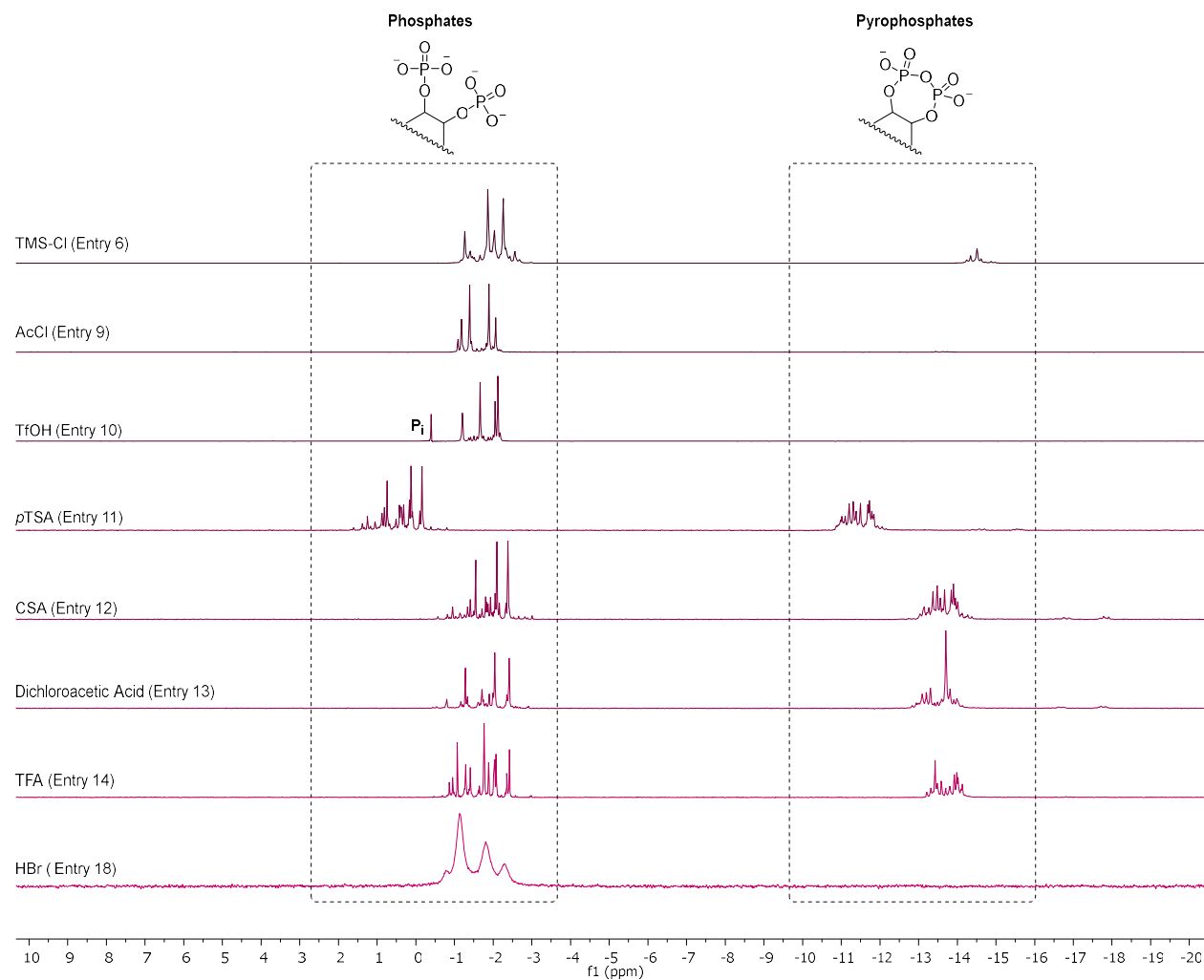
4.1.2. Towards hydrolysis of ITPP to InsP₆

Supplementary Table 5 Ring-opening screening of ITPP **25**. The reaction outcome was analyzed by CE-qTOF-MS (BGE: NH₄OAc 35 mM pH = 9.7, CE voltage 30 kV, CE current: 23 μA, injection 100 mbar, 15 s (30 nL)) and ³¹P{¹H}-NMR. ¹No complete conversion was observed. ²Dephosphorylation was observed by ³¹P-NMR. ³Based on ³¹P{¹H}-NMR the 1/3'-2' pyrophosphate was hydrolysed.⁴



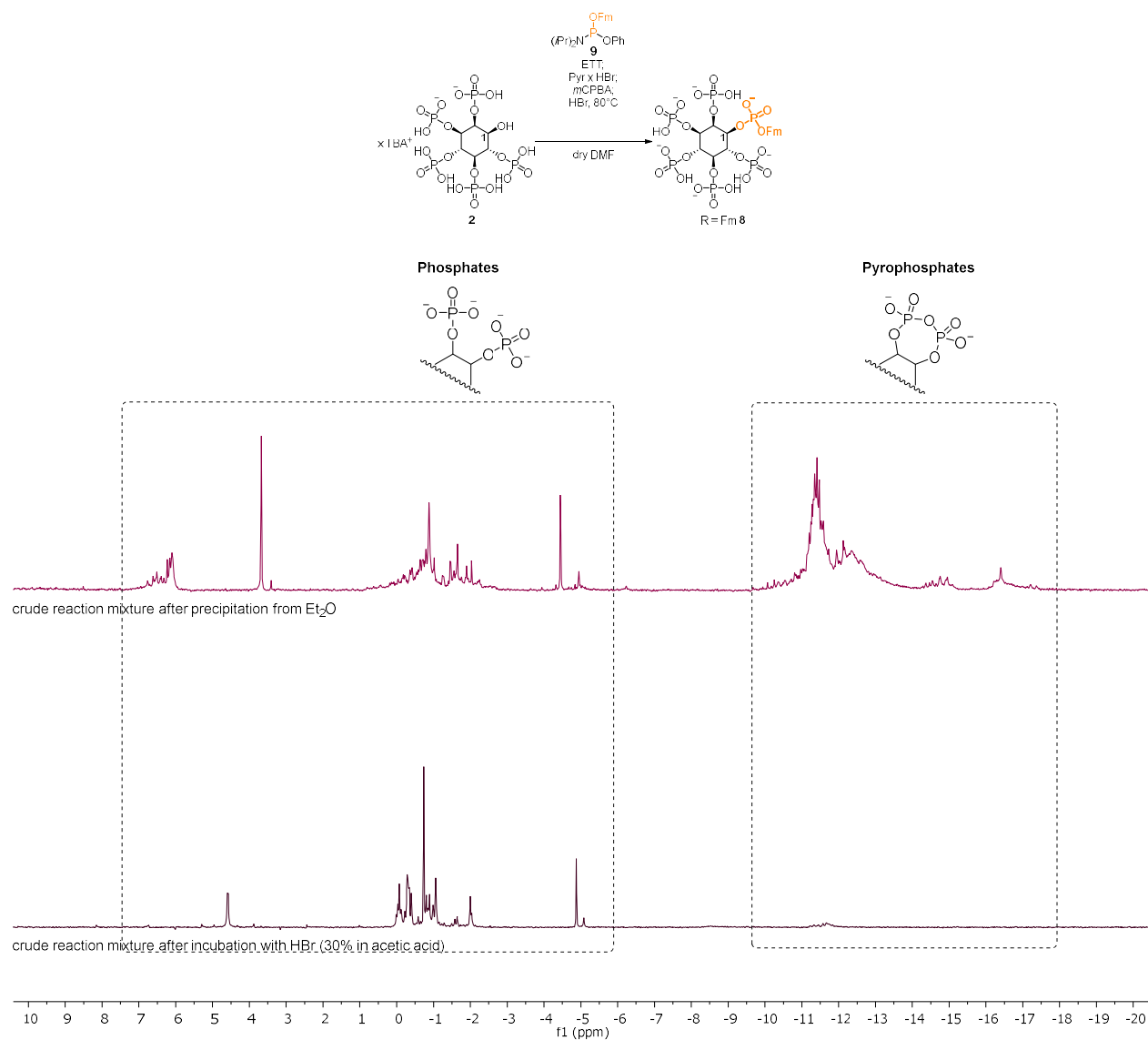
Entry	X	T	Reaction Time	Solvent	Outcome
1	DBU	65°C	60 min	DMSO:water (6:1)	/
2	DABCO	65°C	60 min	DMSO:water (6:1)	/
3	Morpholine	65°C	60 min	DMSO:water (6:1)	/
4	Hydrazine	65°C	60 min	DMSO:water (6:1)	/
5	NaI	65°C	60 min	DMSO:water (6:1)	/
6	TMS-Cl	65°C	60 min	DMSO:water (6:1)	Complete ring-opening ¹
7	AcCl	0°C to RT	overnight	MeOH	Complete ring-opening
8	AcCl	0°C to RT	overnight	BnOH:DMSO:water (5:2.5:1)	/
9	AcCl	65°C	60 min	DMSO:water (6:1)	Complete ring-opening ¹
10	TfOH	65°C	60 min	DMSO:water (6:1)	Complete ring-opening ²
11	<i>p</i> TSA × H ₂ O	65°C	60 min	DMSO:water (6:1)	Partial ring-opening ³
12	CSA	65°C	60 min	DMSO:water (6:1)	Partial ring-opening ³
13	Dichloroacetic acid	65°C	60 min	DMSO:water (6:1)	Partial ring-opening ³
14	TFA	65°C	60 min	DMSO:water (6:1)	Partial ring-opening ³
15	AgOTf	65°C	60 min	DMSO:water (6:1)	/
16	Amberlite 15 H ⁺ -Form	65°C	60 min	DMSO:water (6:1)	/
17	Conc. HCl (40vol%)	65°C	2 days	water	/
18	HBr (30% in acetic acid, 30vol%)	80°C	2 h	water	Complete ring-opening
19	Phenyl Phosphate (6 equiv.)	RT	overnight	water	/
20	Benzyl Phosphate (6 equiv.)	RT	overnight	water	/

4.2. NMR monitoring of the hydrolysis of ITPP



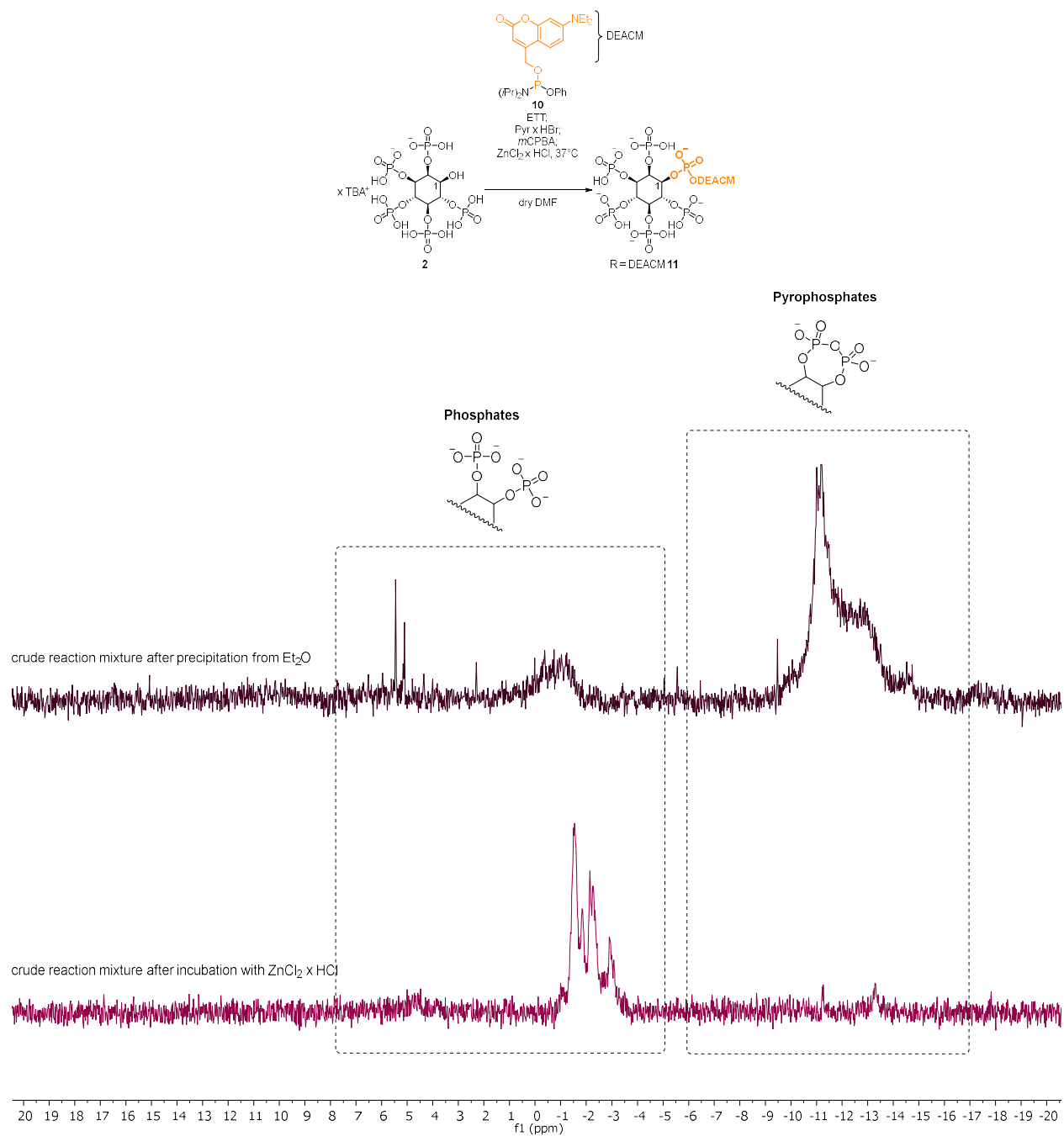
Supplementary Figure 4 ^{31}P -NMR comparison of (partial)-ring openings of ITPP **25** with organic acids in DMSO:water (see Supplementary Figure 5 Entry 6 and 9 – 14).

4.3. ^{31}P -NMR monitoring of the hydrolysis of 1-Fm-InsP₆ 8



Supplementary Figure 5 The ring opening of cyclic 1-Fm-InsP₆ derivatives by HBr treatment was monitored by ^{31}P -NMR.

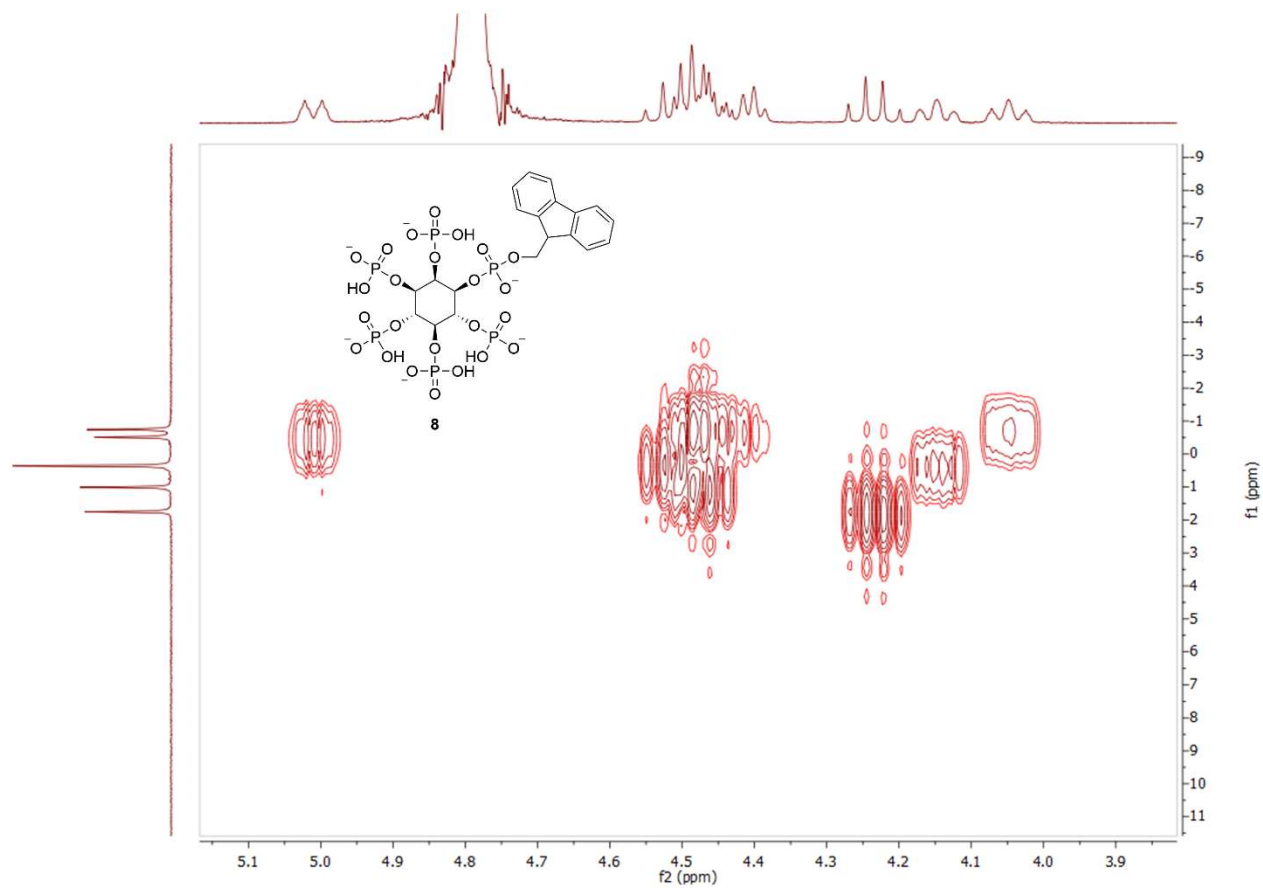
4.4. ^{31}P -NMR monitoring of the hydrolysis of 1-DEACM-InsP₆ 11



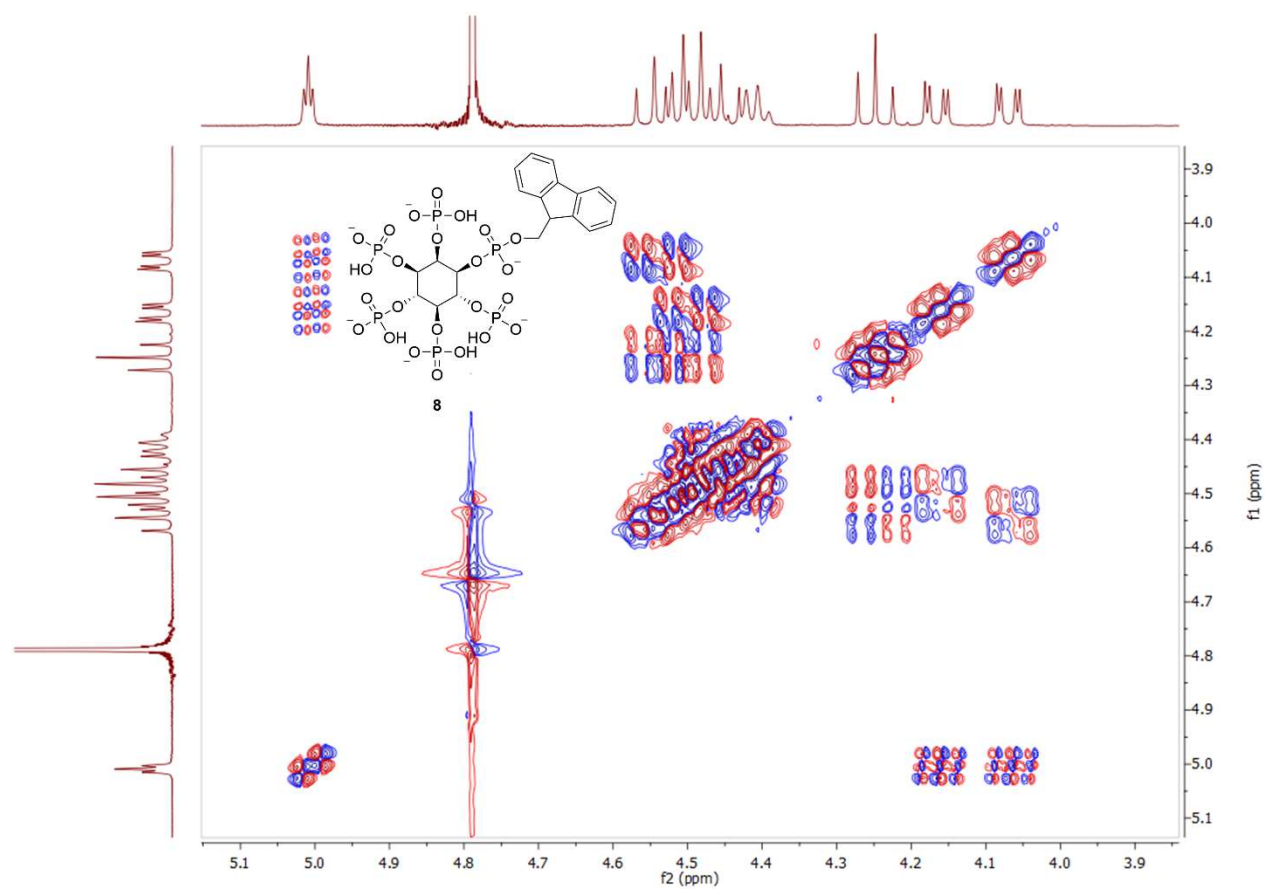
Supplementary Figure 6
 ^{31}P -NMR.

The ring opening of cyclic 1-DEACM-InsP₆ derivatives by $\text{ZnCl}_2 \times \text{HCl}$ treatment was monitored by

4.5. 2D-NMR of 1-Fm-InsP₆ **8**



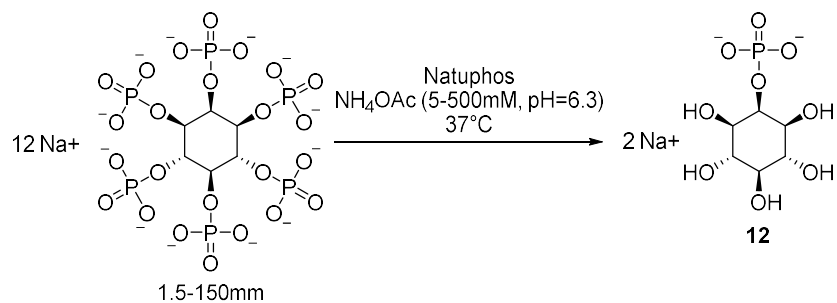
Supplementary Figure 7 ³¹P-¹H-HMBC-NMR of 1-Fm-InsP₆ **8**.



Supplementary Figure 8 $\{^{31}\text{P}\}$ - ^1H - ^1H -DQF-COSY-NMR of 1-Fm-InsP₆ **8**.

5. Natuphos digest of InsP₆

Supplementary Table 6 Screening for optimal conditions for the Natuphos (10500 U/mL) digest of InsP₆. 5vol% of Natuphos (10500 U/mL) solution (270 mg/mL) was used. ¹Conditions used in literature.⁹

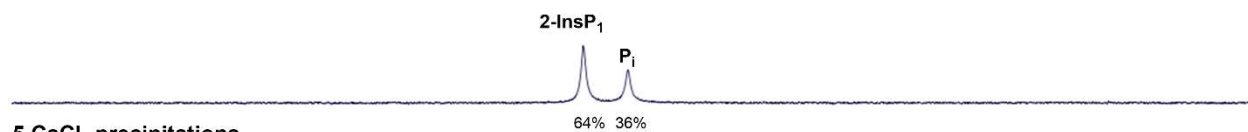


Entry	[InsP ₆]	[NH ₄ OAc]	Main Product after 10 min
1	150 mM	500 mM	2-InsP ₁
2	150 mM	50 mM	2-InsP ₁
3	150 mM	5 mM	2-InsP ₁
4	150 mM	50 mM	2-InsP ₁
5 ¹	15 mM	50 mM	2-InsP ₁
6	1.5 mM	50 mM	2-InsP ₁

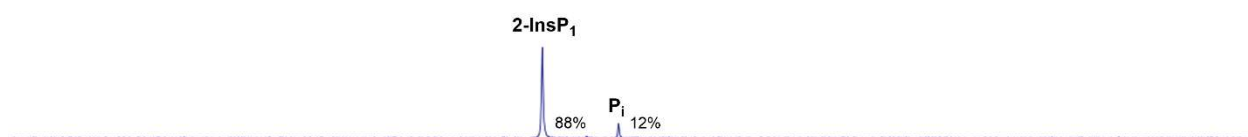
5.1. Purification of 2-InsP₁ *via* precipitation monitored *via* ³¹P-NMR monitoring

³¹P{¹H}-NMR's of the Ca₃(PO₄)₂ precipitation progress

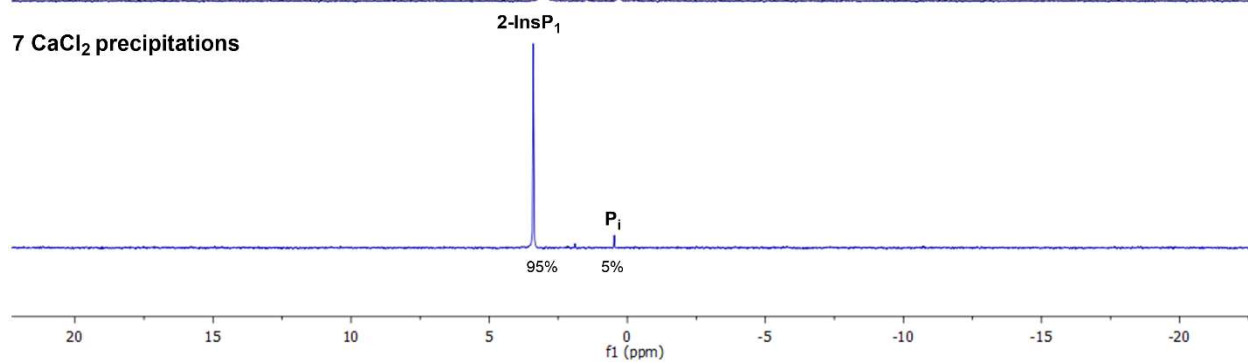
3 CaCl₂ precipitations



5 CaCl₂ precipitations

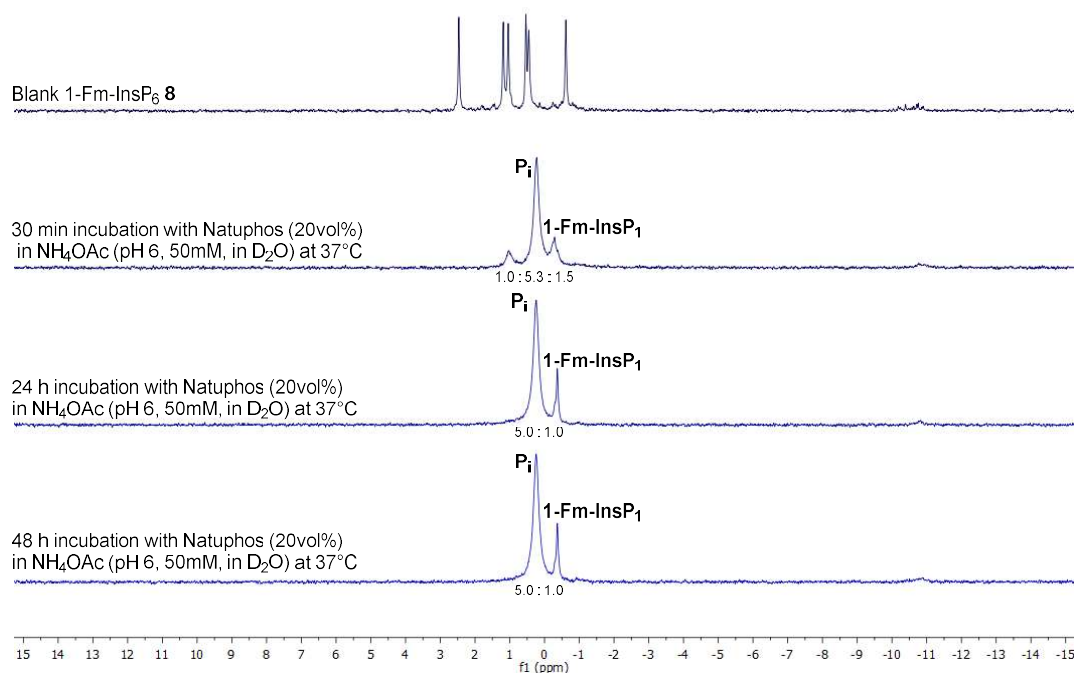


7 CaCl₂ precipitations



Supplementary Figure 9 The Ca₃(PO₄)₂ precipitation progress during 2-InsP₁ purification was monitored by ³¹P{¹H}-NMR.

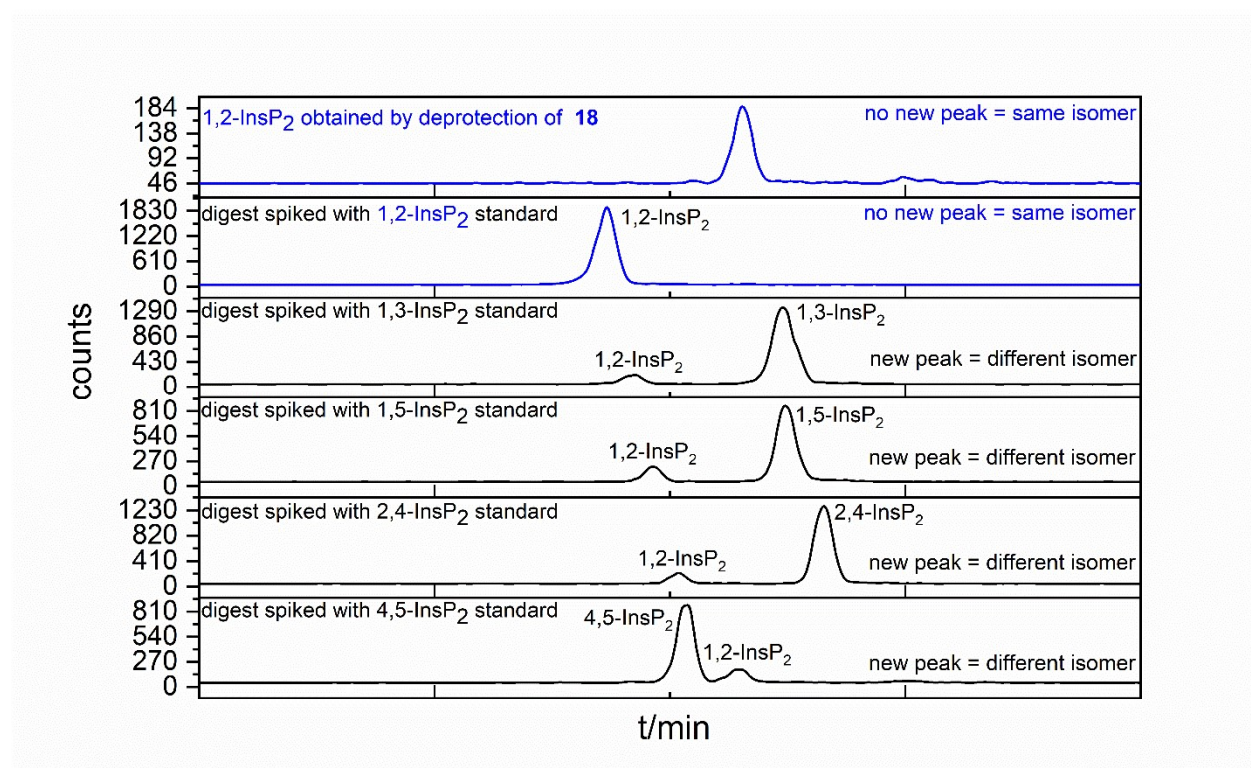
5.2. Natuphos digest of 1-Fm-InsP₆ **8** monitored *via* ³¹P-NMR



Supplementary Figure 10 The Natuphos (10500 U/mL) digest of 1-Fm-InsP₆ **8** to 1-Fm-InsP₁ **13** and inorganic phosphate was monitored by ³¹P{¹H}-NMR

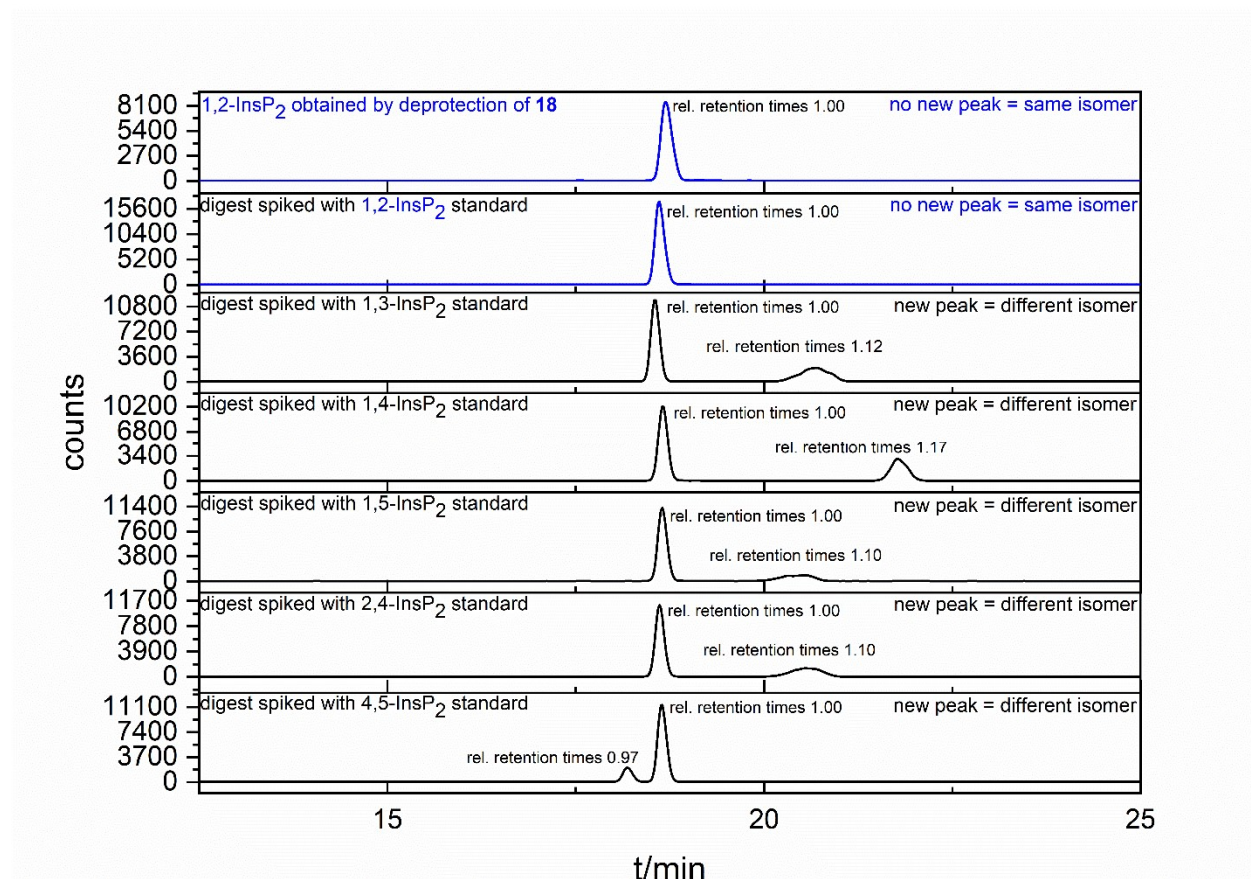
6. Identification of the formed InsP_2 isomers obtained by dephosphorylation of 1-Fm- InsP_6 8

6.1. by Natuphos – spiking experiments with InsP_2 standards



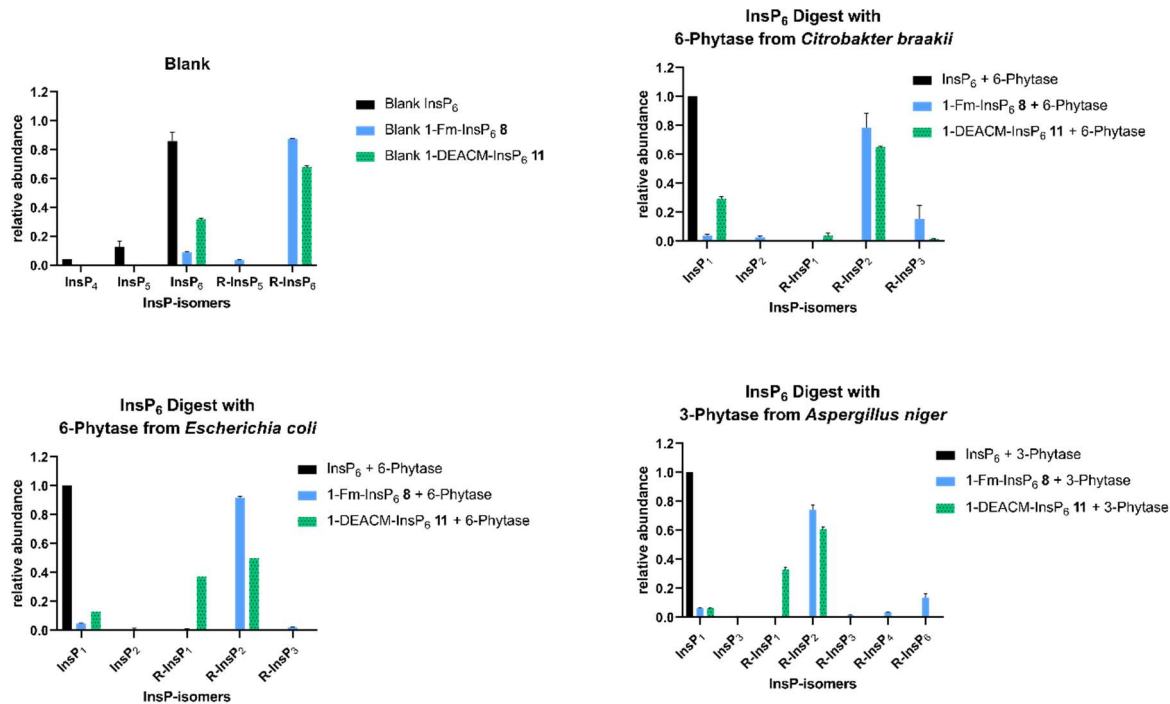
Supplementary Figure 11 CE-QQQ-MS (BGE: NH_4OAc 35 mM pH = 9.7, CE voltage: 30 kV, CE current: 23 μA , injection: 100 mbar, 10 s (20 nL)) analysis of the digest of 1-Fm- InsP_6 **8** by Natuphos (10500 U/mL) after subsequent basic deprotection. Spiking experiments with defined isomers allowed to identification of 1,2- InsP_2 .¹⁰

6.2. by 6-Phytase from *Escherichia coli* (7500 U/mL) – spiking experiments with InsP₂ standards



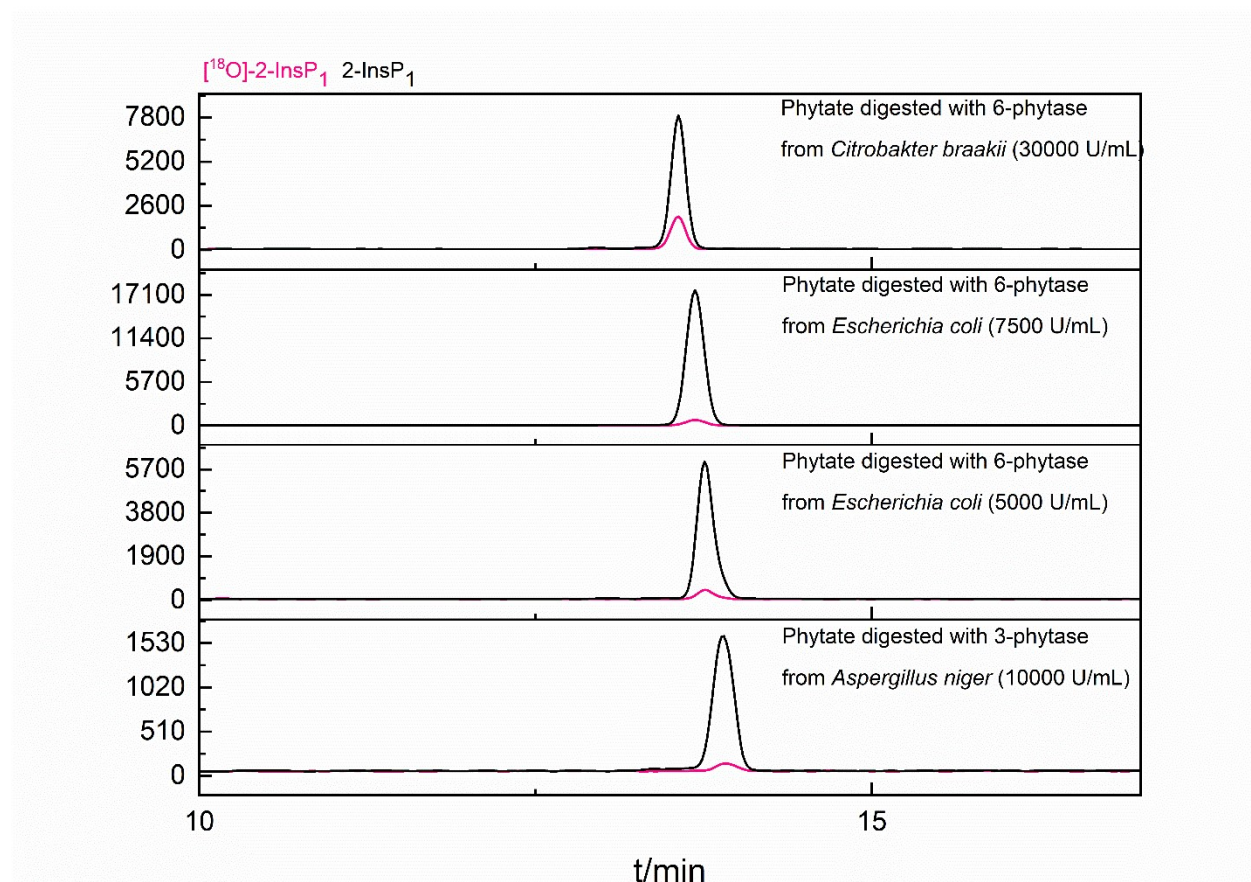
Supplementary Figure 12 CE-MS (BGE: NH₄OAc 35 mM pH = 9.7, CE voltage: 30 kV, CE current: 23 μ A, injection: 100 mbar, 10 s (20 nL)) analysis of the digest of 1-Fm-InsP₆ **8** with 6-Phytase from *Escherichia coli* (7500 U/mL) after subsequent basic deprotection. Spiking experiments with defined isomers allowed to identification of 1,2-InsP₂.¹⁰

7. Phytase Screen



Supplementary Figure 13 CE-qTOF-MS (BGE: NH₄OAc 35 mM pH = 9.7, CE voltage: 30 kV, CE current: 23 μA, injection: 100 mbar, 15 s (30 nL)) analysis of the digest of modified InsP₆ derivatives with different phytases (6-phytase from *Citrobacter braaki*, 30000 U/mL; 6-Phytase from *Escherichia coli*, 5000 U/mL; 3-phytase from *Asperigillus niger*, 10000 U/mL).

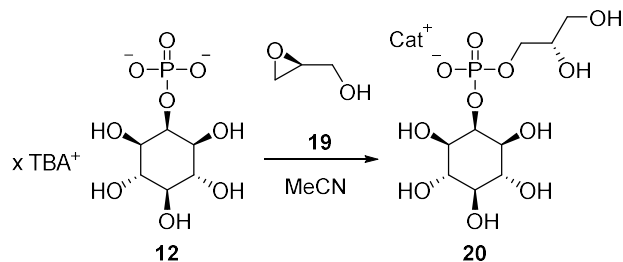
7.1. Identification of the formed InsP_1 via spiking experiments



Supplementary Figure 14 CE-MS (BGE: NH_4OAc 35 mM pH = 9.7, CE voltage: 30 kV, CE current: 23 μA , injection: 100 mbar, 10 s (20 nL)) spiking experiments with ^{18}O -2-InsP₁ allowed identification of 2-InsP₁ as product of the InsP_6 digests of the tested phytases.

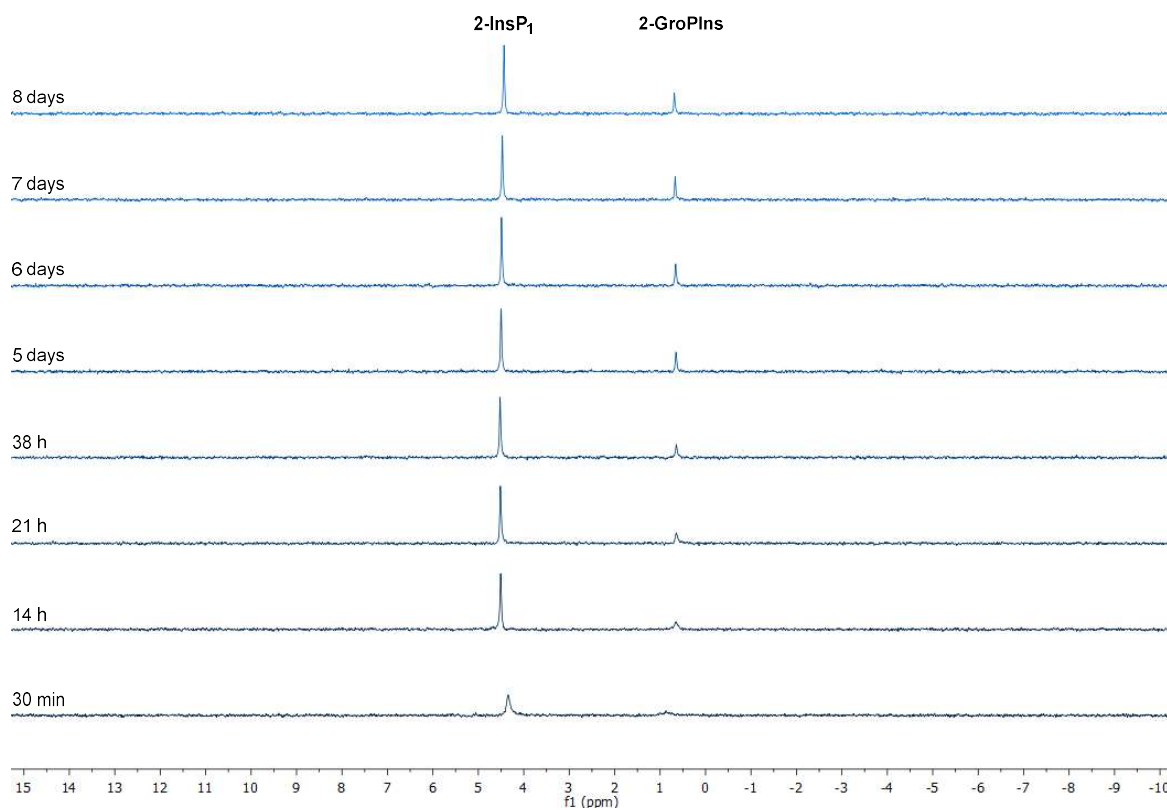
8. Co(III) catalysis screening

Supplementary Table 7 Reaction screening of the ring opening of *S*-(-)-glycidol with 2-InsP₁ **12** under Co(III) catalysis using JACOBSENS catalyst **19**.¹Based on ³¹P{¹H}-NMR integration. ²2-InsP₁ × 1.3 TBA was poorly soluble in MeCN. ³Based on ¹H-NMR 0.6 equiv. TBA-OH were present.



Entry	TBA ⁺	S-(-)-glycidol	19	t	12:20 ¹
1 ²	1.3 equiv.	1.0 equiv.	10mol%	Overnight	/
2 ²	1.3 equiv.	1.5 equiv.	10mol%	Overnight	/
3 ²	1.3 equiv.	2.0 equiv.	10mol%	Overnight	/
4 ²	1.3 equiv.	1.0 equiv.	5mol%	Overnight	/
5 ²	1.3 equiv.	1.5 equiv.	5mol%	Overnight	/
6 ²	1.3 equiv.	2.0 equiv.	5mol%	Overnight	1.0:0.16
7	1.5 equiv.	0.7 equiv.	35mol%	8 days	1.0:0.3
8	1.5 equiv.	1.0 equiv.	35mol%	2 days	1.0:0.3
9	1.5 equiv.	1.4 equiv.	35mol%	8 days	1.0:0.3
10	1.5 equiv.	0.7 equiv.	21mol%	8 days	1.0:0.3
11	1.5 equiv.	0.7 equiv.	7mol%	8 days	1.0:0.4
12 ³	2.6 equiv.	1.0 equiv.	10mol%	Overnight	1.0:0.1
13 ³	2.6 equiv.	1.5 equiv.	10mol%	Overnight	1.0:0.1
14 ³	2.6 equiv.	2.0 equiv.	10mol%	overnight	1.0:0.1

8.1. NMR monitoring for extend reaction times



Supplementary Figure 15 $^{31}\text{P}\{^1\text{H}\}$ -NMR of the ring opening of *S*-(-)-glycidol with 2-InsP₁ **12** catalysed by **19** (Supplementary Table 7, Entry 11).

9. Literature

- 1 G. R. Fulmer, A. J. M. Miller, N. H. Sherden, H. E. Gottlieb, A. Nudelman, B. M. Stoltz, J. E. Bercaw and K. I. Goldberg, *Organometallics*, 2010, **29**, 2176–2179.
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- 3 T. Weinrich, M. Gränz, C. Grünewald, T. F. Prisner and M. W. Göbel, *European J. Org. Chem.*, 2017, **2017**, 491–496.
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- 6 L. P. C. Nielsen, C. P. Stevenson, D. G. Blackmond and E. N. Jacobsen, *J. Am. Chem. Soc.*, 2004, **126**, 1360–1362.
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- 10 G. Liu, E. Riemer, R. Schneider, D. Cabuzu, O. Bonny, C. A. Wagner, D. Qiu, A. Saiardi, A. Strauss, T. Lahaye, G. Schaaf, T. Knoll, J. P. Jessen and H. J. Jessen, *RSC Chem. Biol.*, 2023, **4**, 300–309.

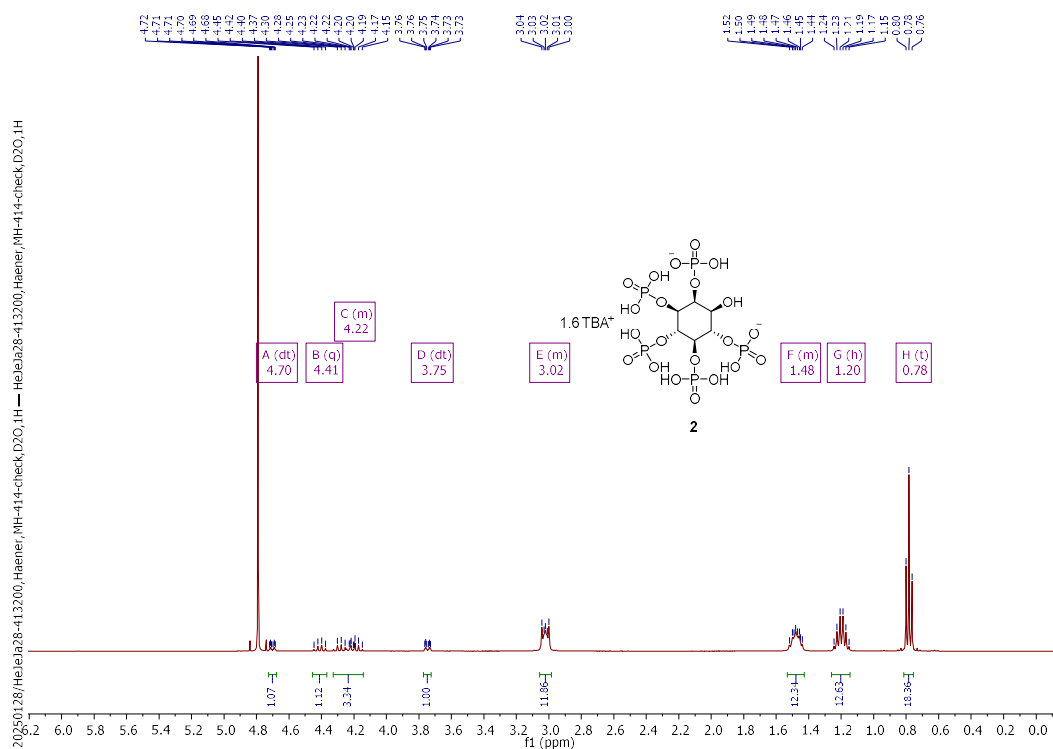
10.1. Blank

10.5. Dephosphorylation of phytate with 3-phytase from *Aspergillus niger* (10000 U/mL)

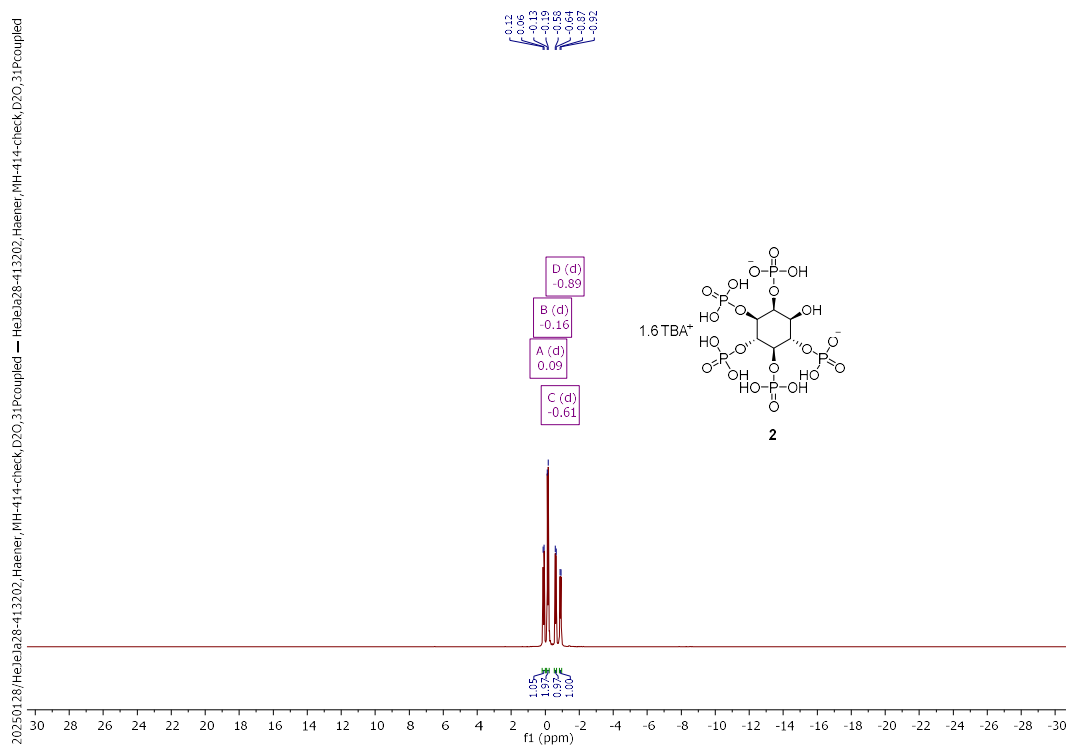
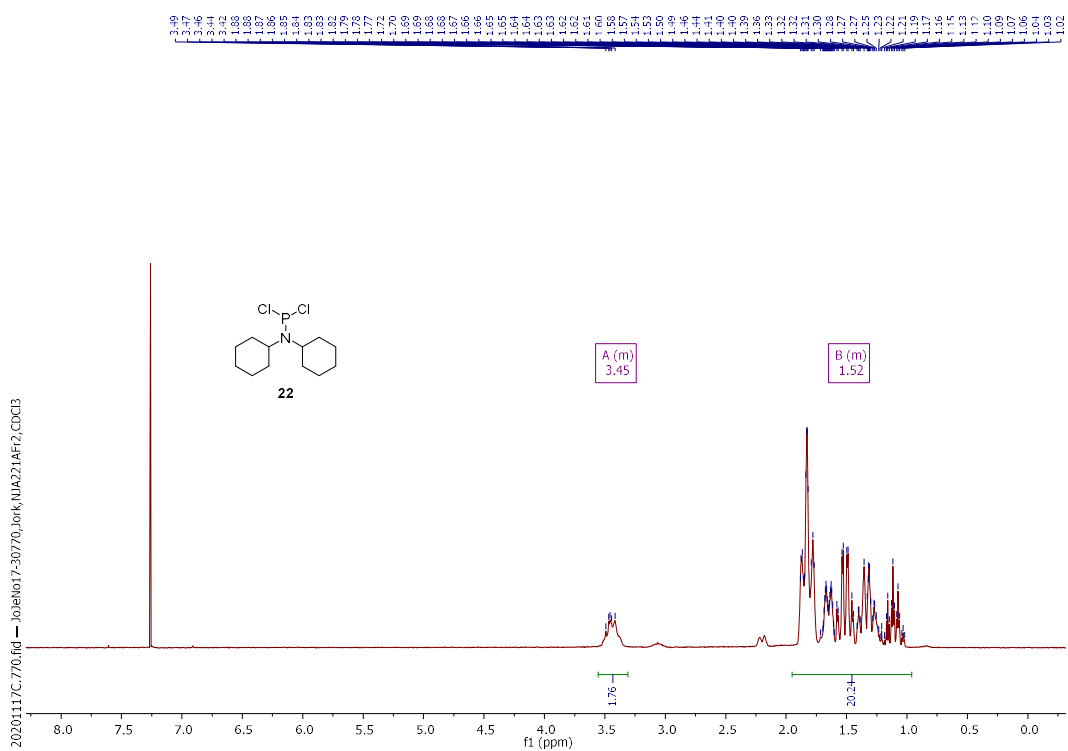
Supplementary Table 12 CE-qTOF-MS (CE-qTOF-MS (BGE: NH₄OAc 35 mM pH = 9.7, CE voltage: 30 kV, CE current: 23 μA, injection: 100 mbar, 15 s (30 nL)) analysis digest of InsP₆ derivatives with 3-phytase from *Aspergillus niger* (10000 U/mL).

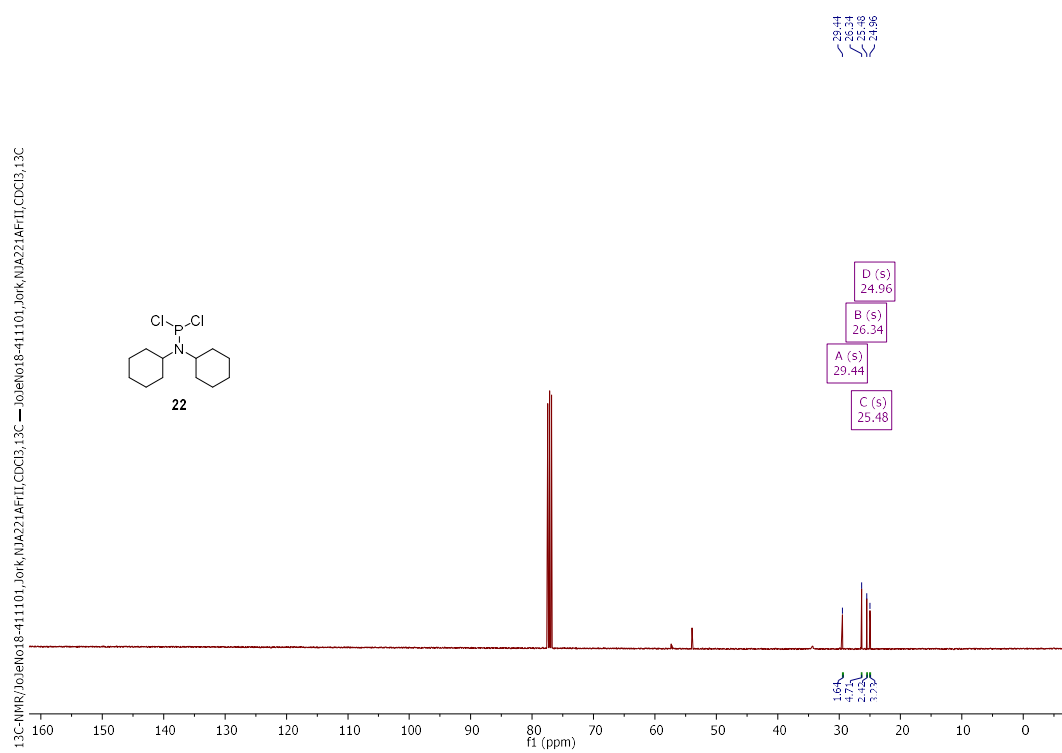
Phytase 21									
InsP ₆			1-Fm-InsP ₆			1-DEACM-InsP ₆			
InsP ₁	1	1	1	0.06391	0.06366	0.06560	0.06079	0.06406	0.06451
InsP ₂									
InsP ₃					0.00530	0.00726			
InsP ₄									
InsP ₅									
InsP ₆									
R-InsP ₁							0.34033	0.30993	0.33751
R-InsP ₂				0.77362	0.74720	0.71100	0.59888	0.62603	0.59798
R-InsP ₃				0.01532	0.01490	0.01496			
R-InsP ₄				0.03641	0.03488	0.03663			
R-InsP ₅									
R-InsP ₆				0.11074	0.13406	0.16455			

11. NMR Spectra

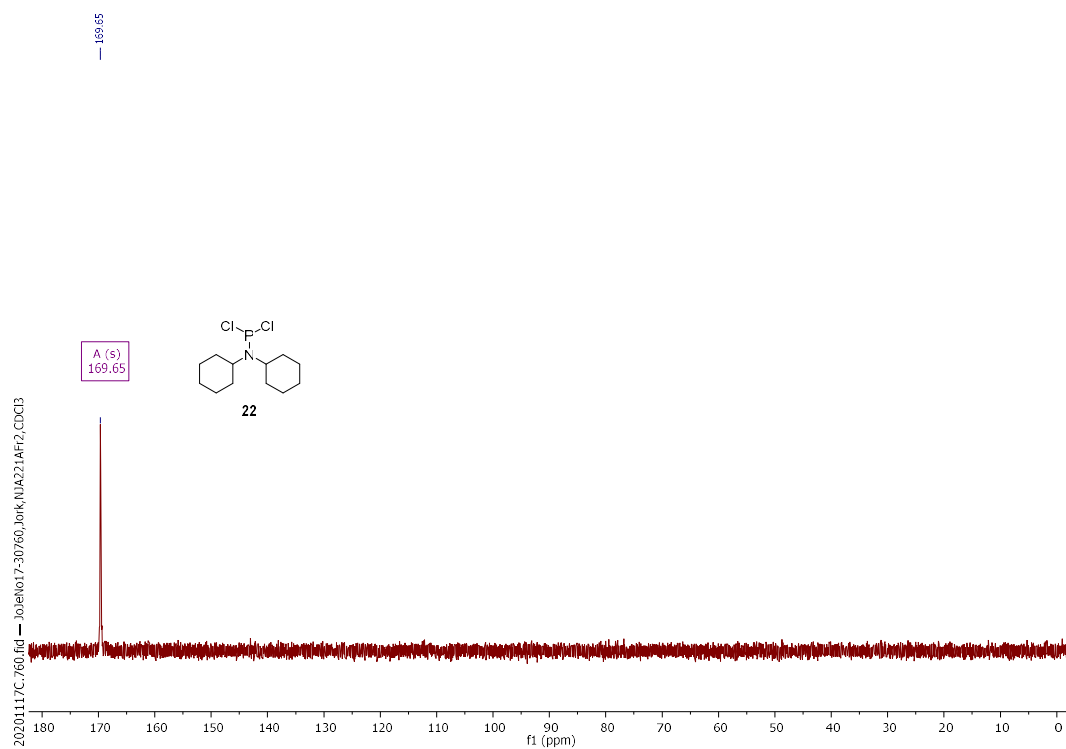


Supplementary Figure 16 ¹H-NMR of the TBA salt of 1-OH-InsP₅ 2.

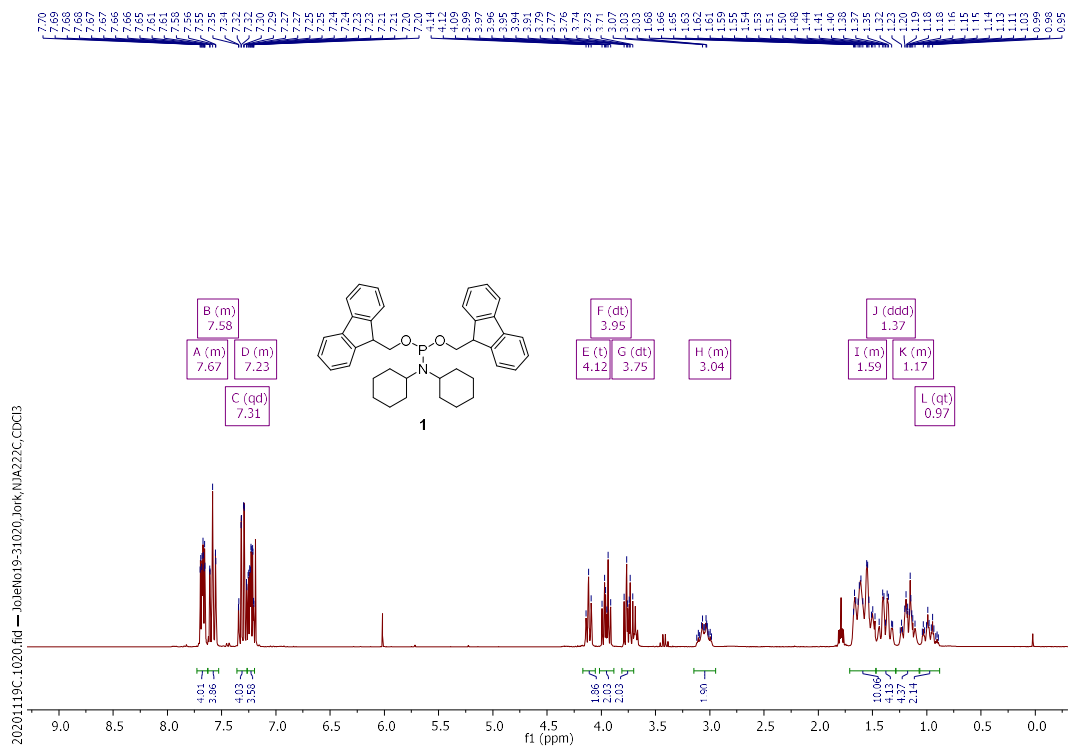
Supplementary Figure 17 ^{31}P -NMR of the TBA-salt of 1-OH-InsP₅ 2.



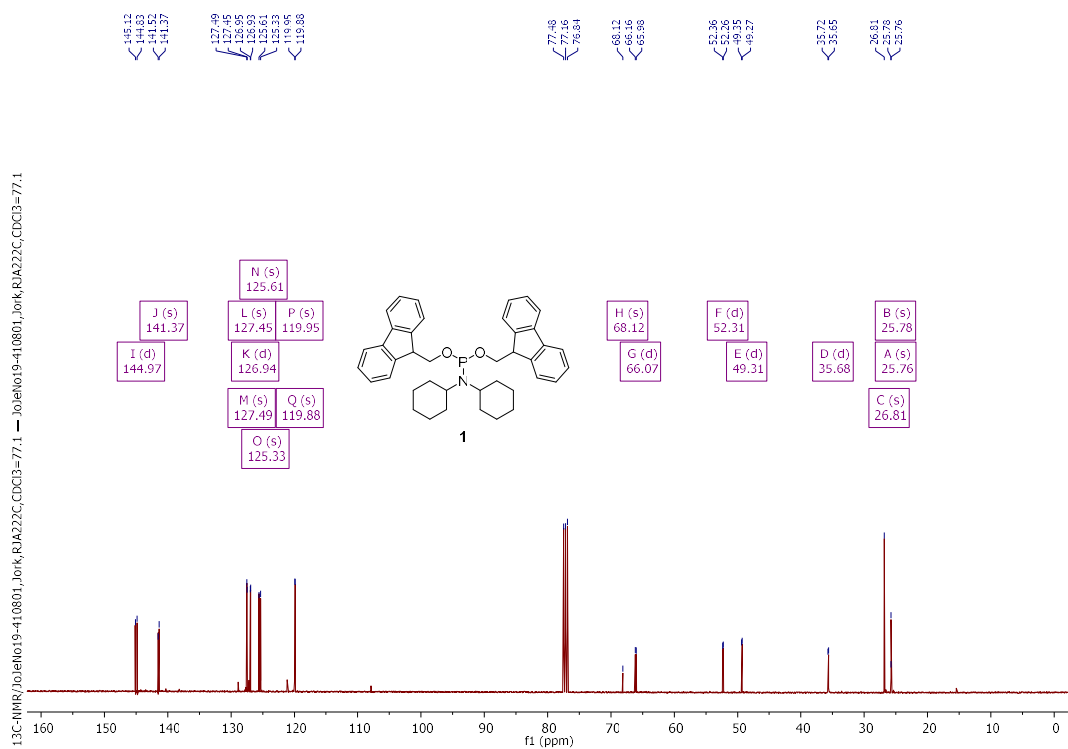
Supplementary Figure 19 ¹³C-NMR of **22**.



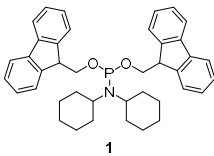
Supplementary Figure 20 ³¹P{¹H}-NMR of **22**.



Supplementary Figure 21 ^1H -NMR of **1**.



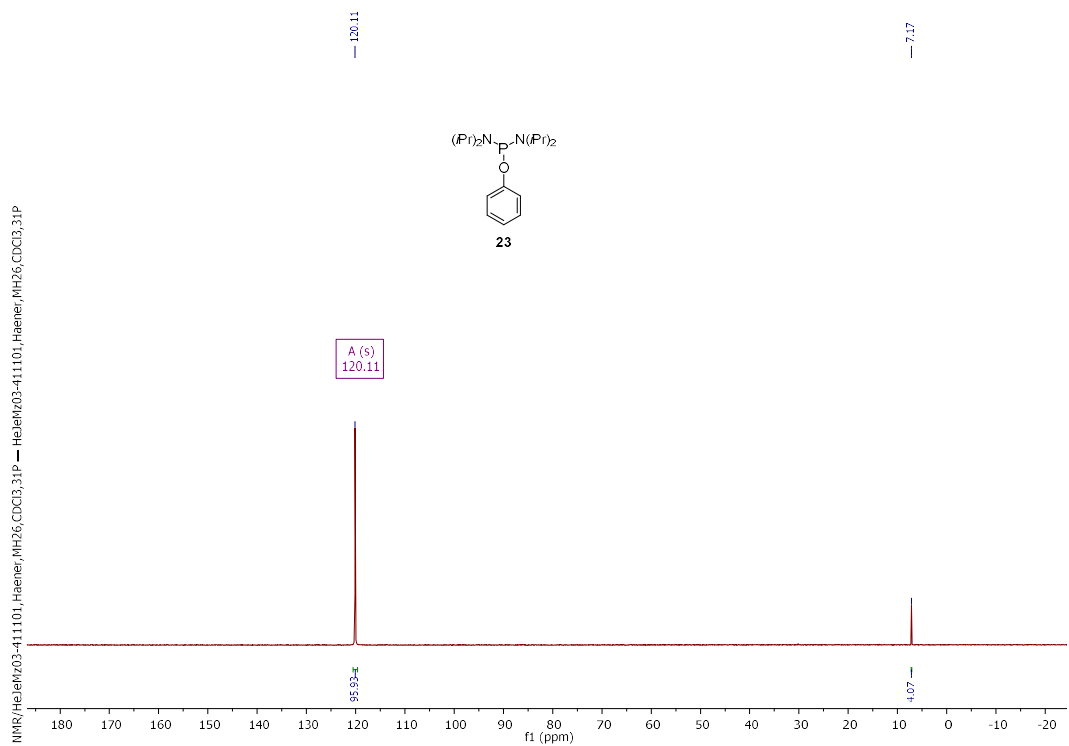
Supplementary Figure 22 ^{13}C -NMR of **1**.



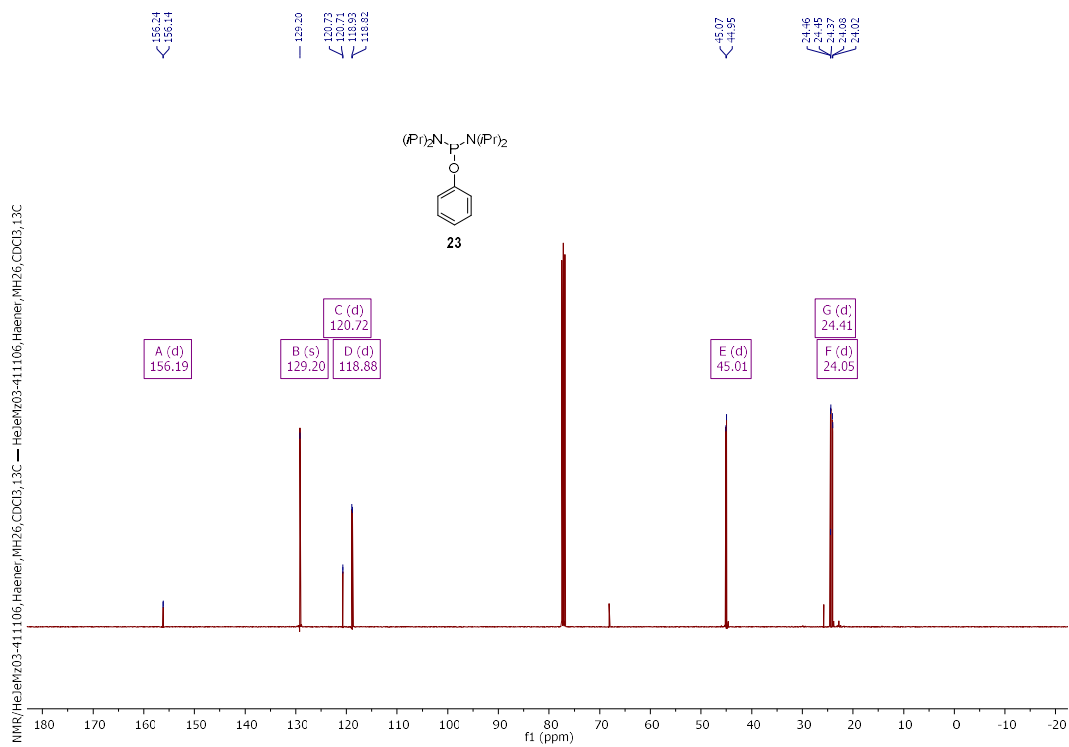
Chemical structure of **23**: CC(C)(C)N(P(C(C)(C)C)C(C)(C)C)Oc1ccccc1

¹H NMR spectrum (CDCl₃) of compound **23**. The spectrum shows peaks at 7.22 (A, dd), 7.04 (B, ddt), 6.90 (C, tt), 3.61 (D, dp), and 1.15 (F, d) ppm. Integration values are 1.03, 1.63, 0.91, 4.04, and 11.99 respectively.

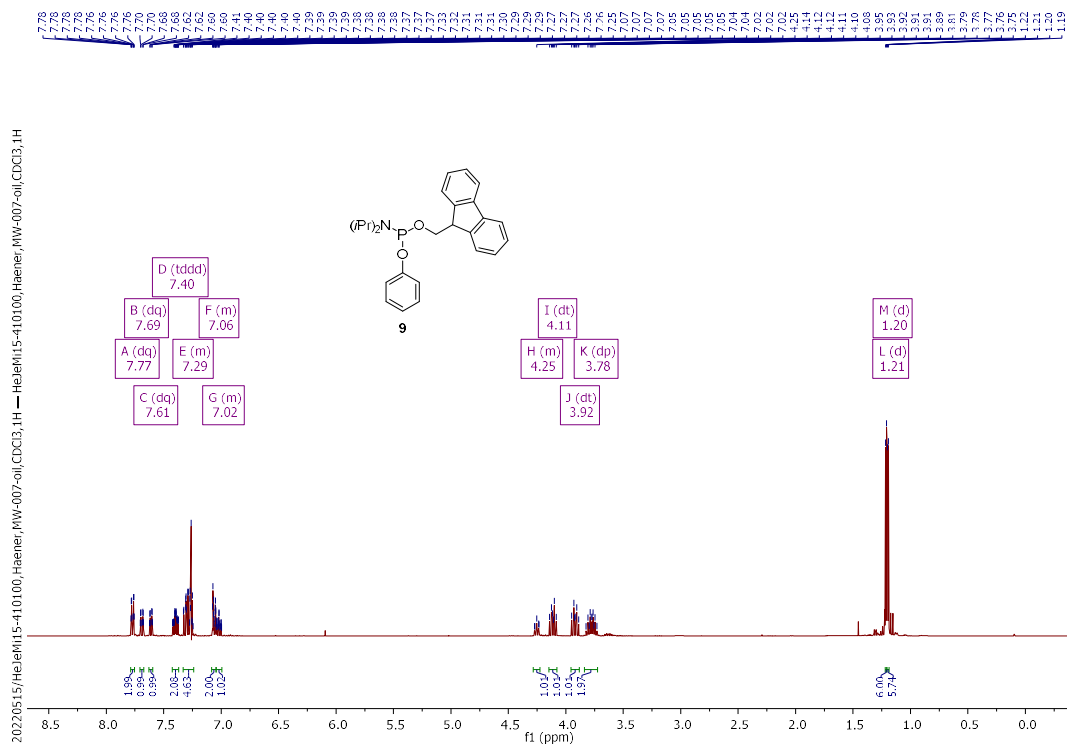
Supplementary Figure 24 ^1H -NMR of **23**.



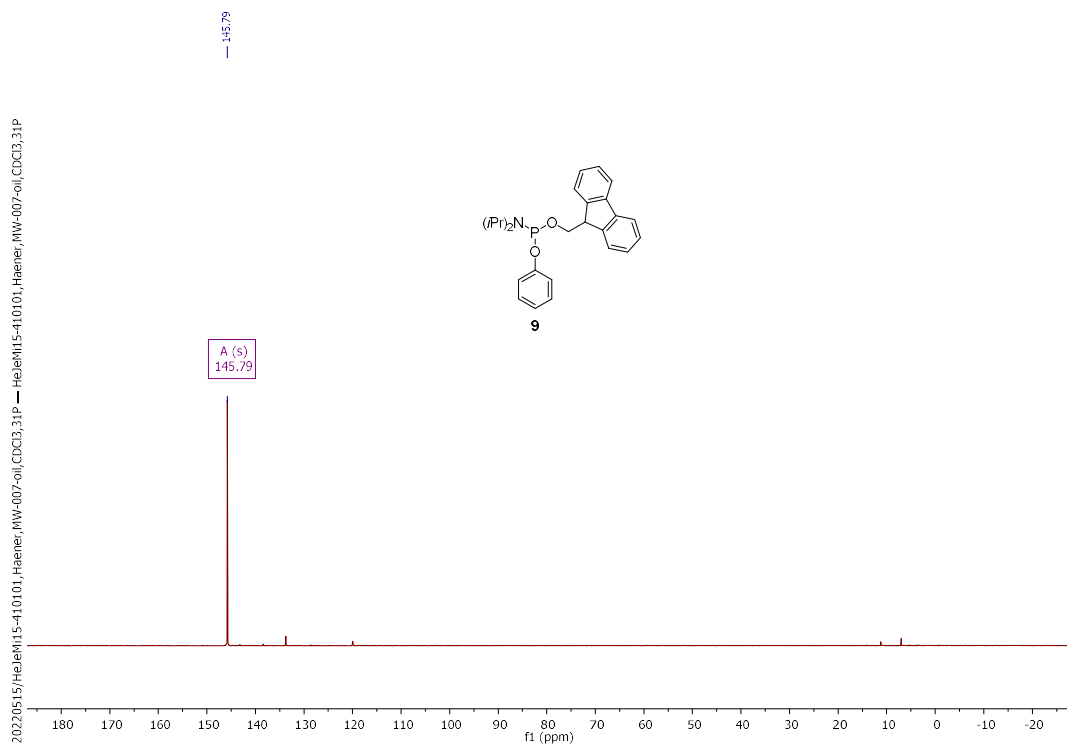
Supplementary Figure 25 $^{31}\text{P}\{^1\text{H}\}$ -NMR of **23**.



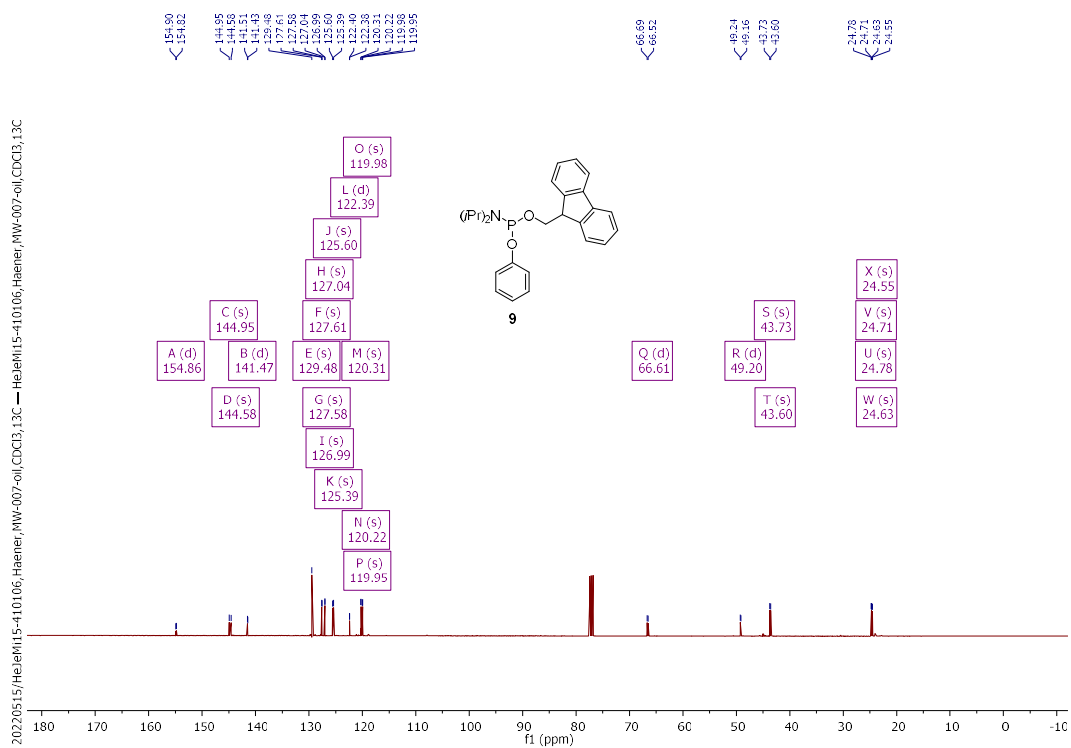
Supplementary Figure 26 ^{13}C -NMR of **23**.



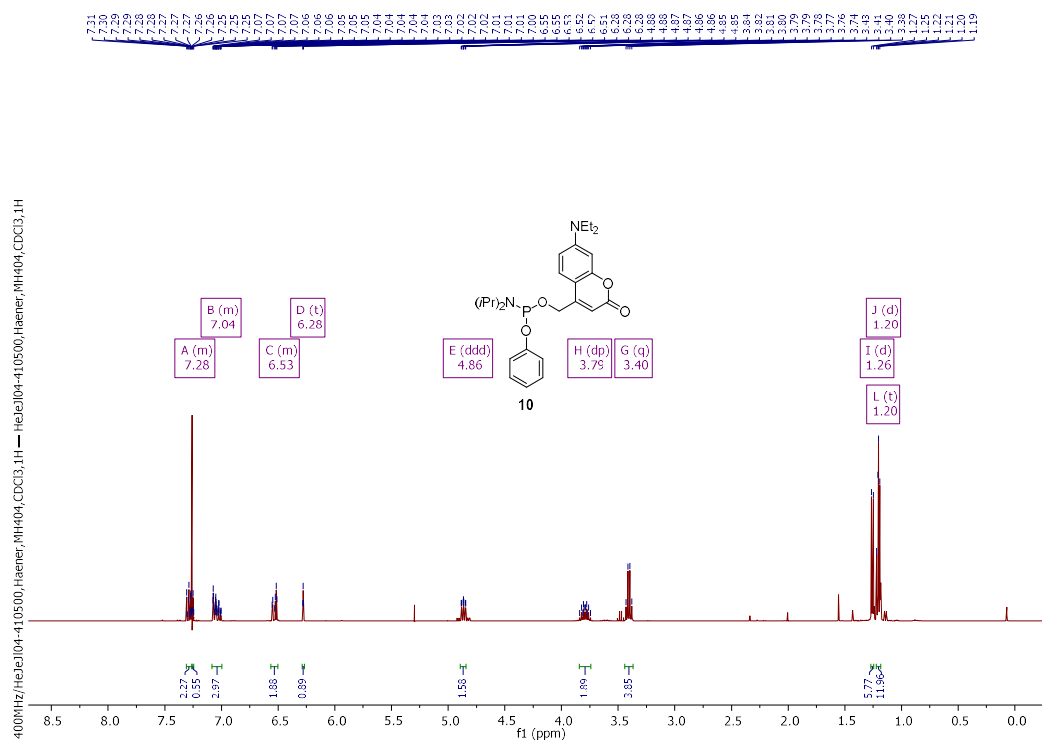
Supplementary Figure 27 ¹H-NMR of **9**.



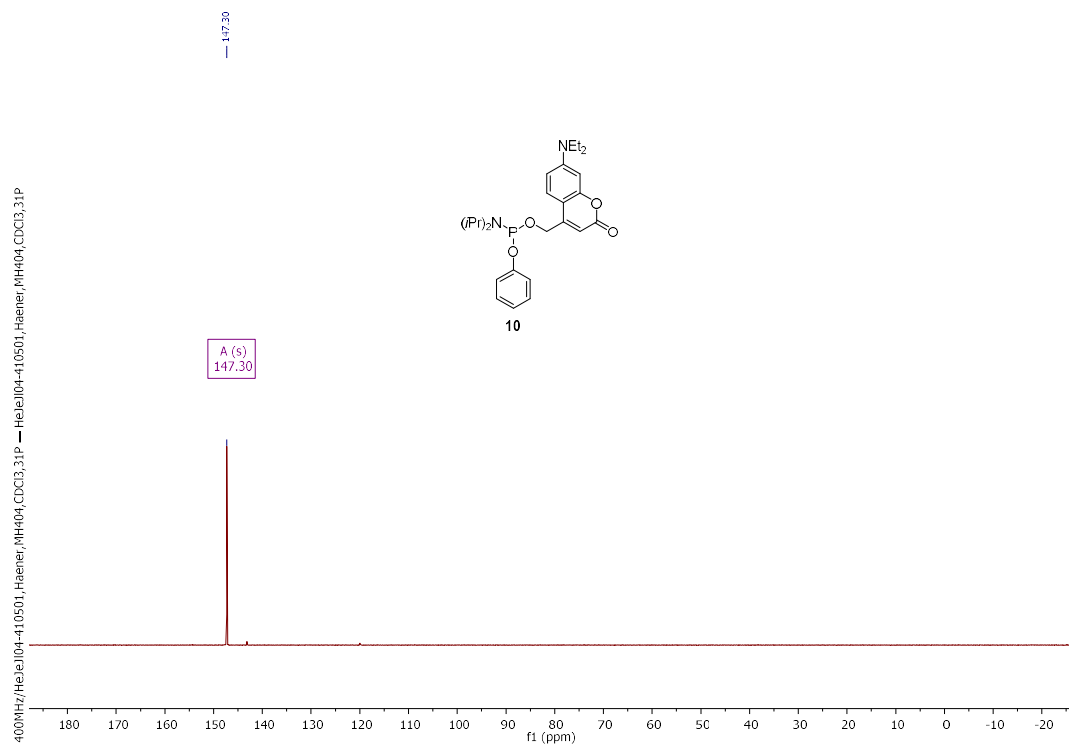
Supplementary Figure 28 ³¹P{¹H}-NMR of **9**.



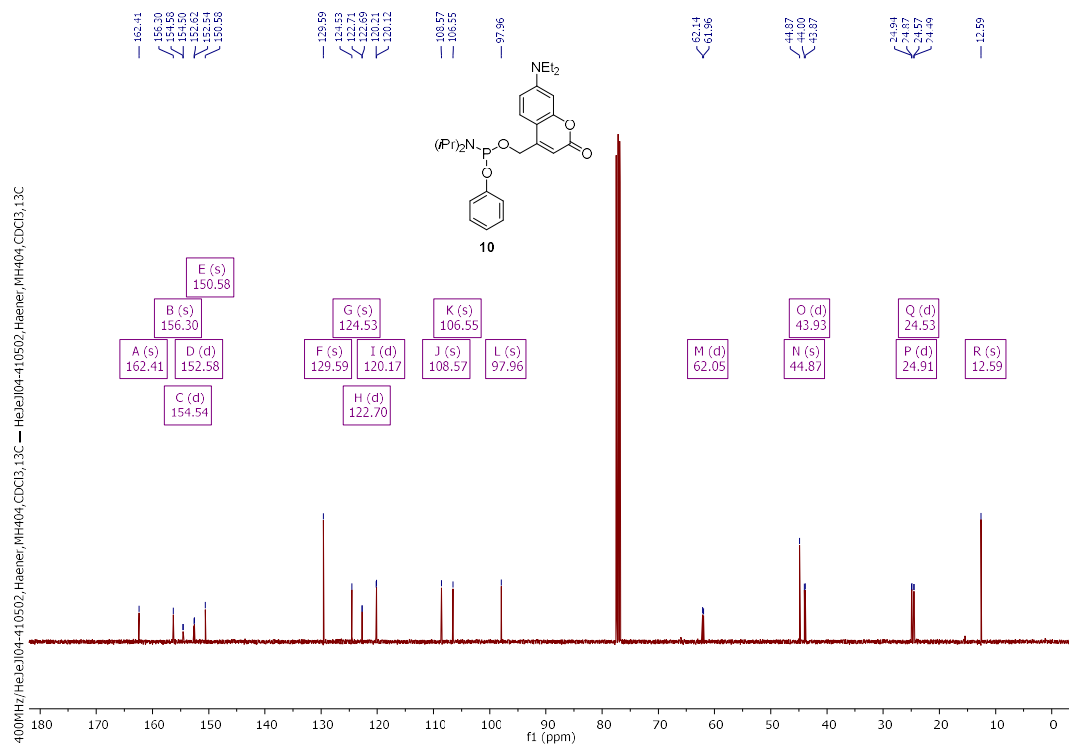
Supplementary Figure 29 ¹³C-NMR of **9**.



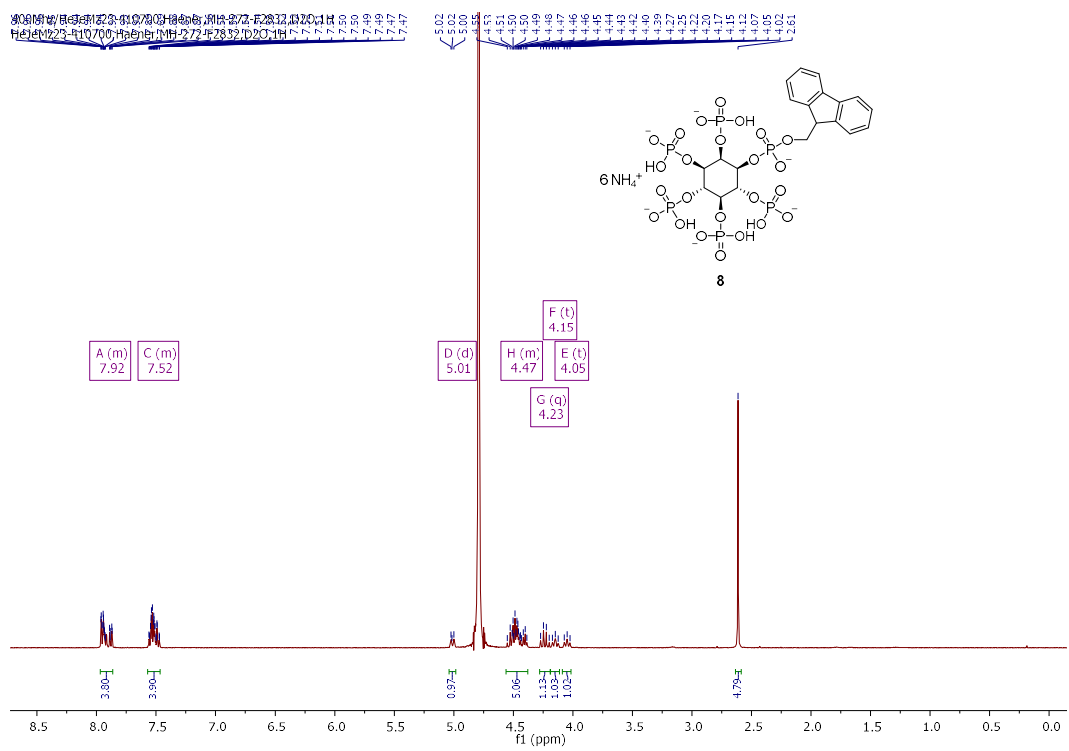
Supplementary Figure 30 ¹H-NMR of **10**.



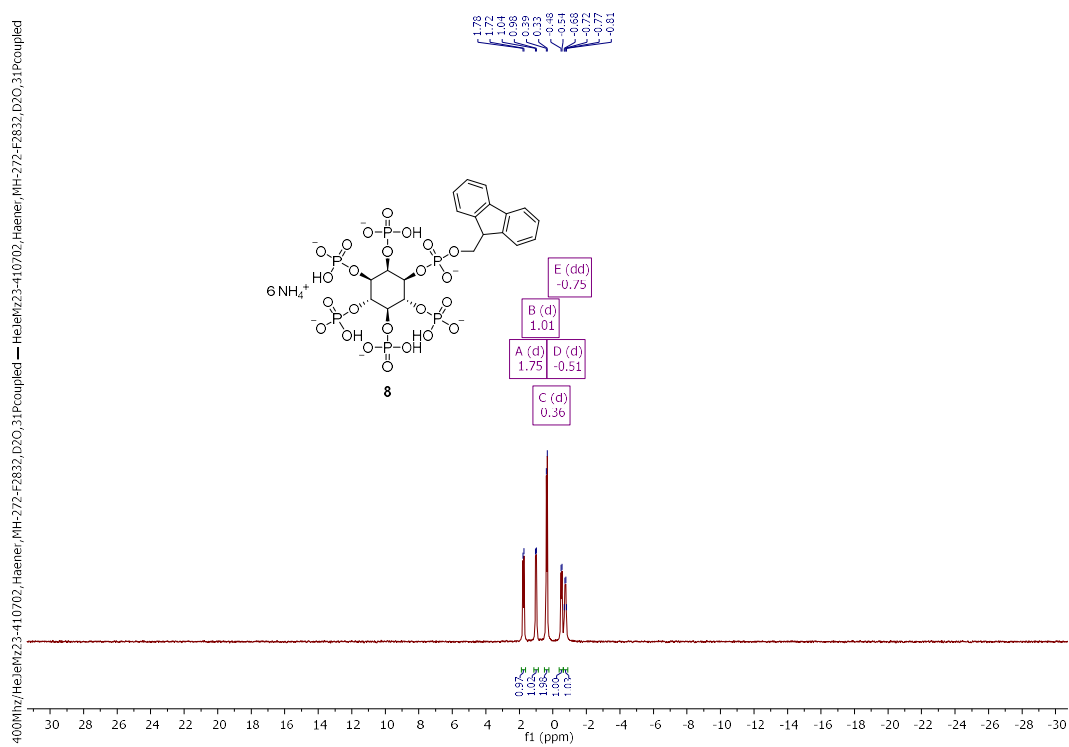
Supplementary Figure 31 $^{31}\text{P}\{^1\text{H}\}$ -NMR of **10**.



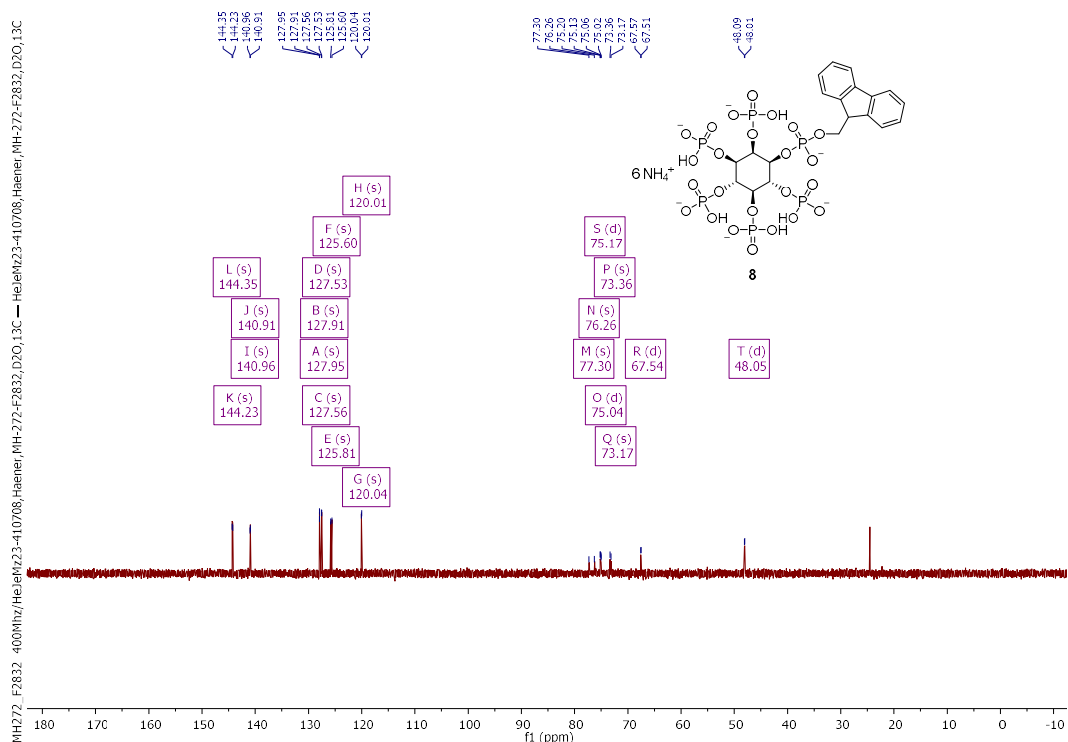
Supplementary Figure 32 ^{13}C -NMR of **10**.



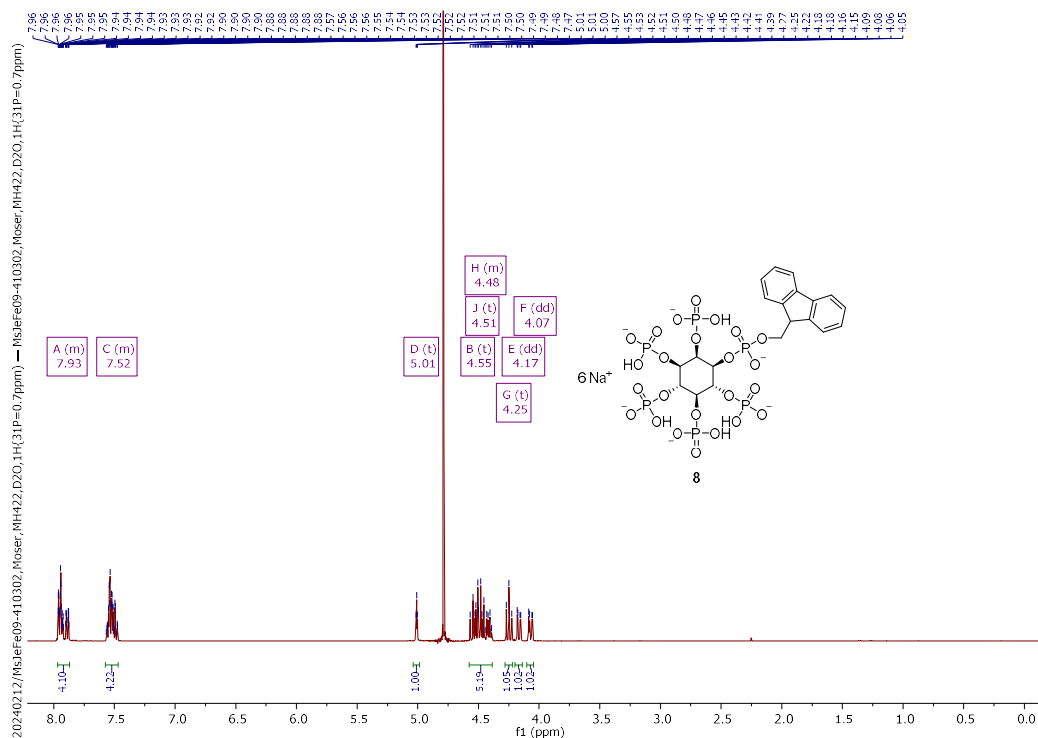
Supplementary Figure 35 ¹H-NMR of 8.



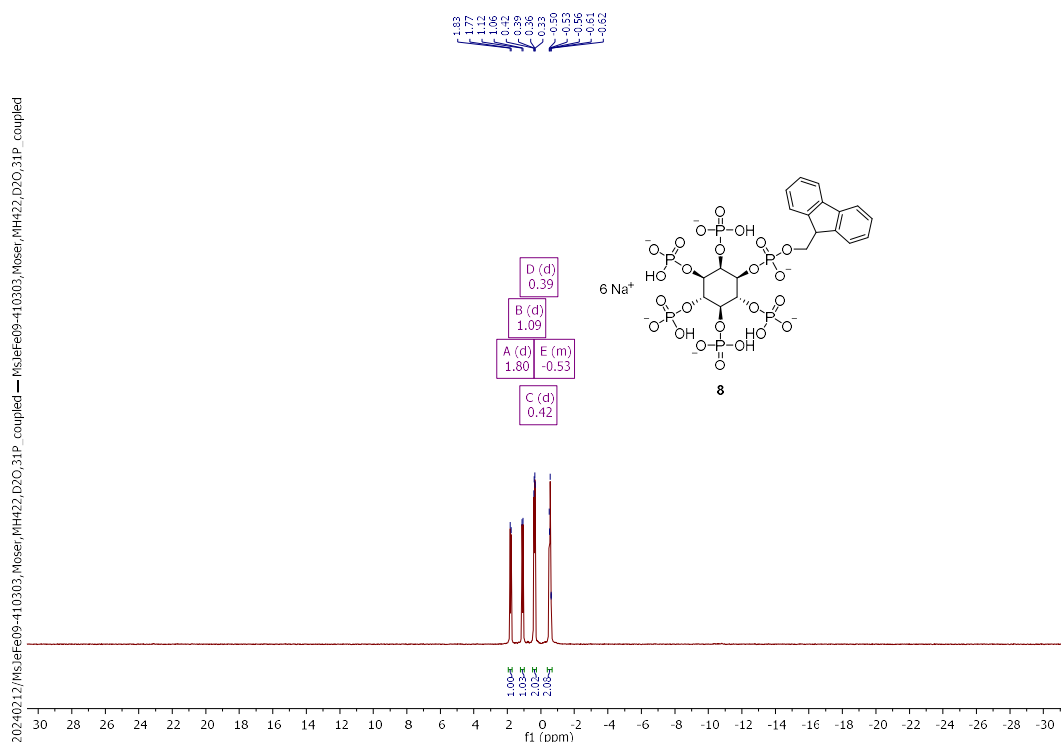
Supplementary Figure 36 ³¹P-NMR of 8.



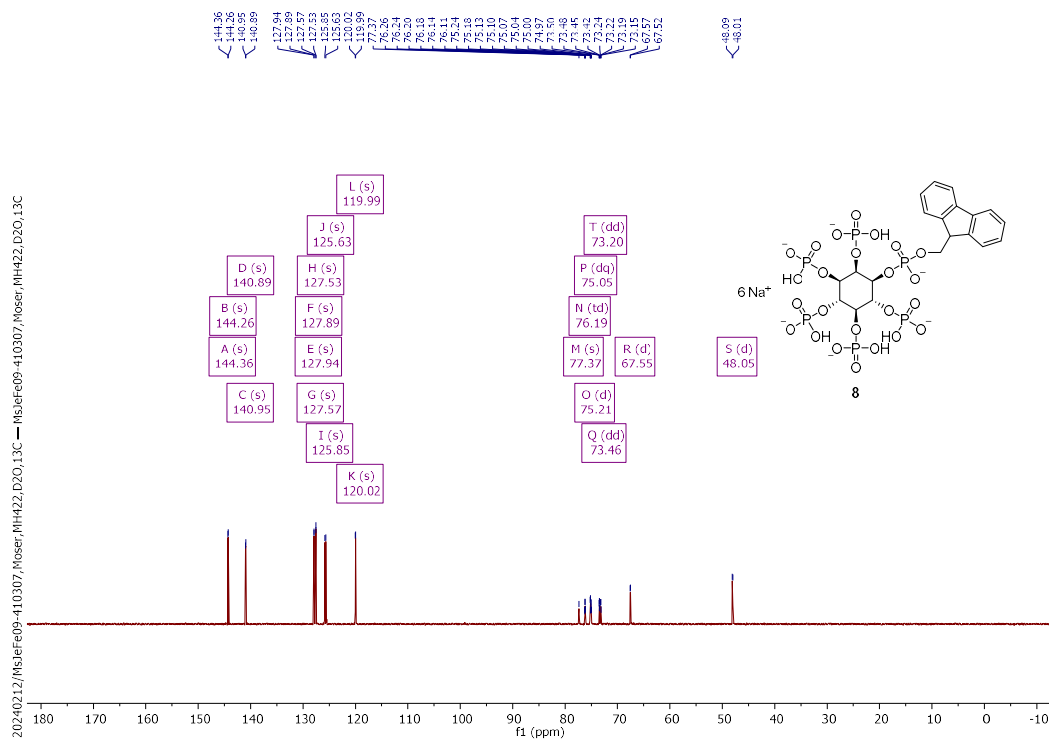
Supplementary Figure 37 ¹³C-NMR of **8**.



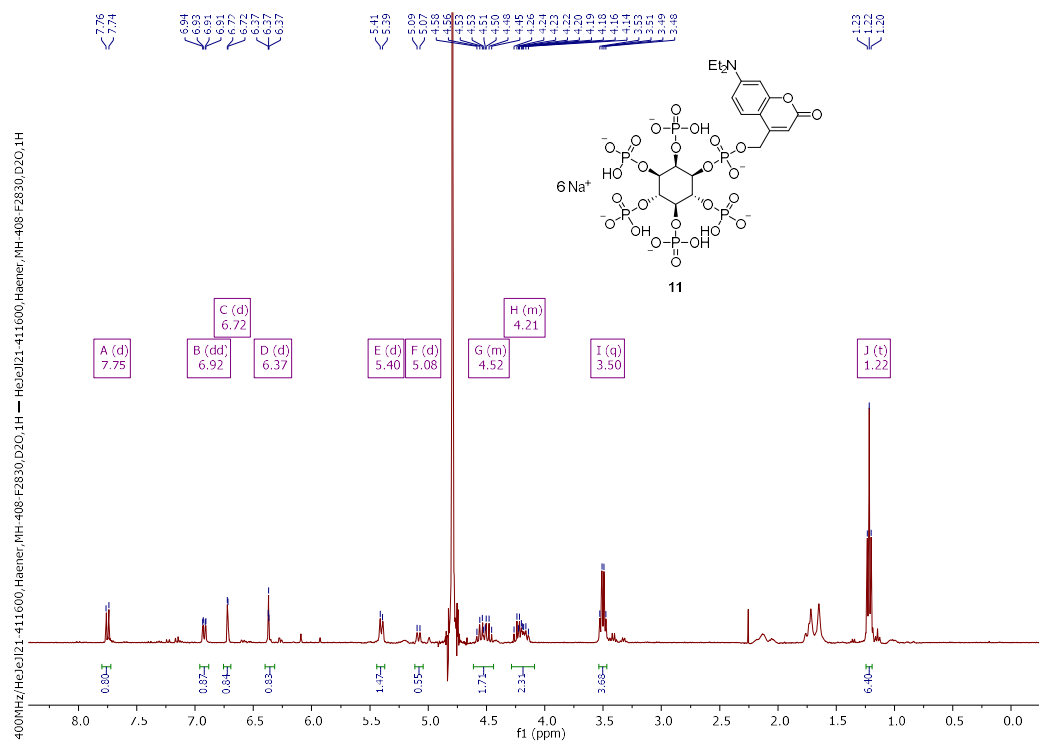
Supplementary Figure 38 ¹H-NMR of **8**.



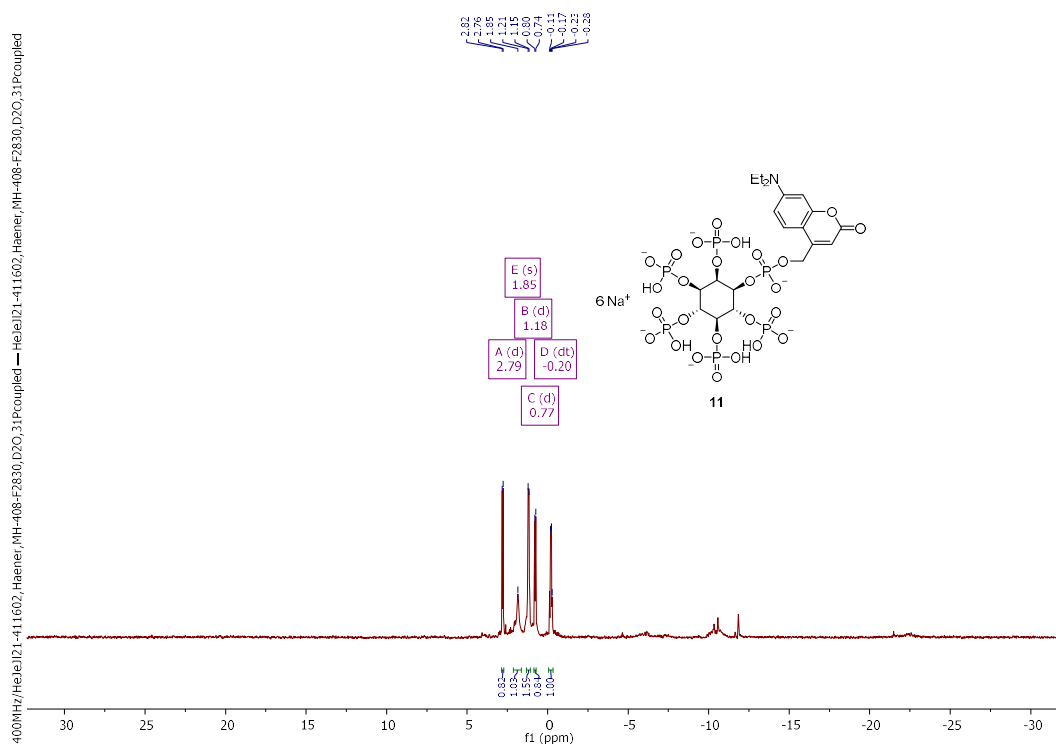
Supplementary Figure 39 ³¹P-NMR of **8**.



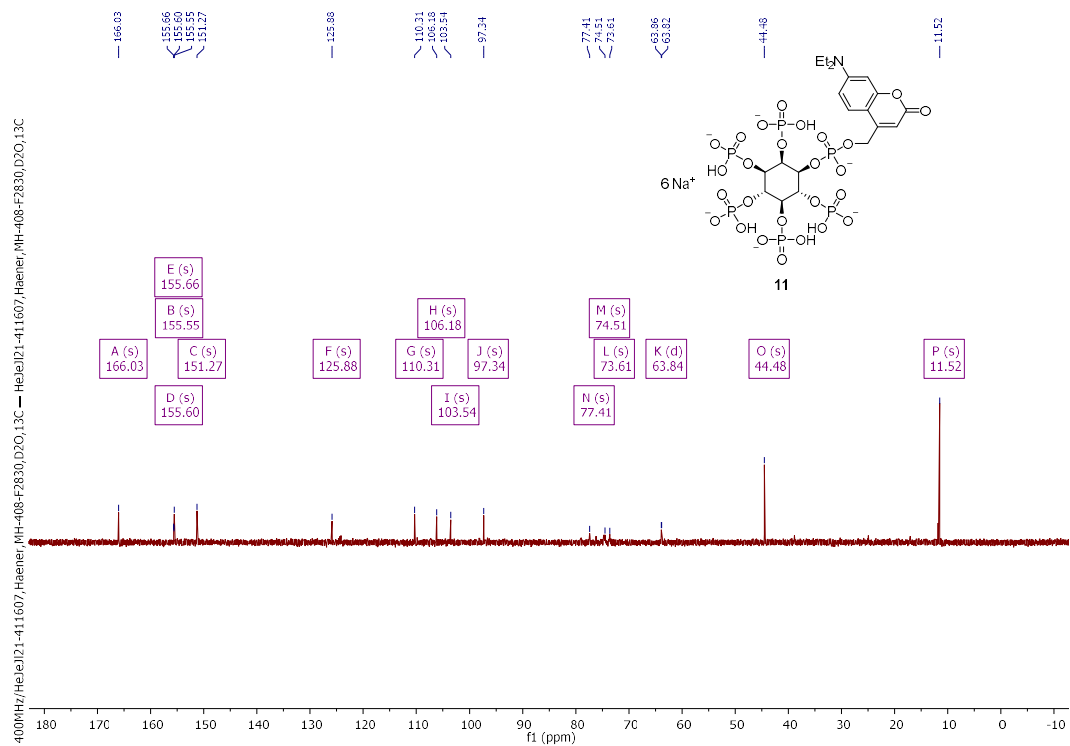
Supplementary Figure 40 ¹³C-NMR of **8**.



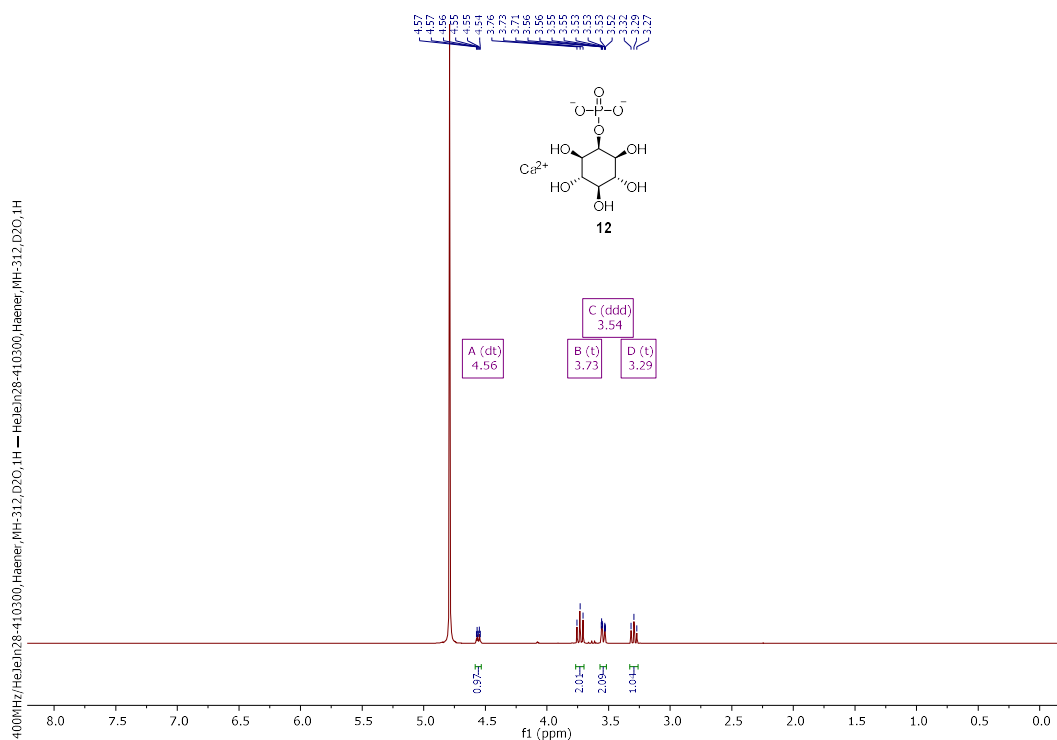
Supplementary Figure 41 ^1H -NMR of **11**.



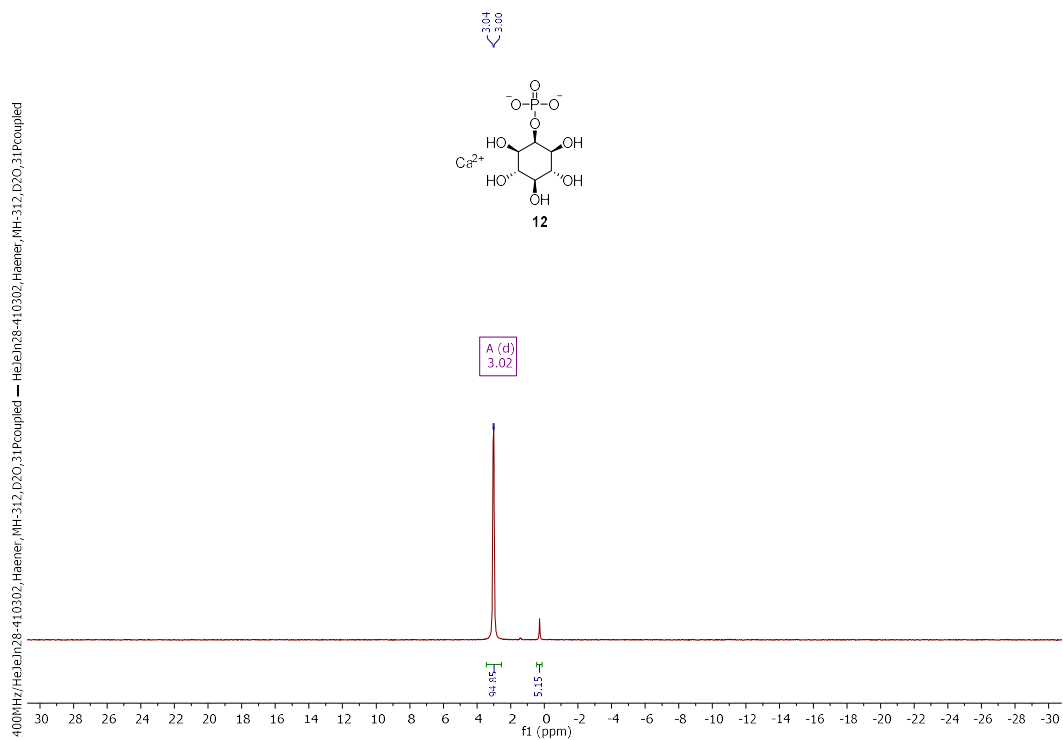
Supplementary Figure 42 ^{31}P -NMR of **11**.



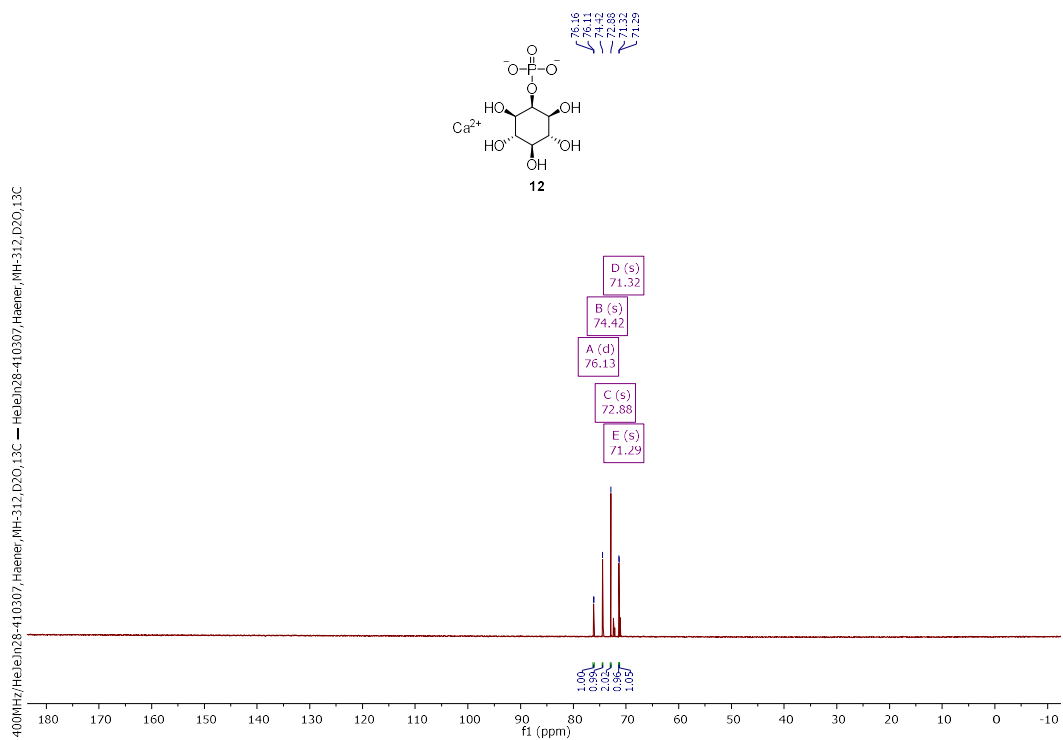
Supplementary Figure 43 ^{13}C -NMR of **11**.



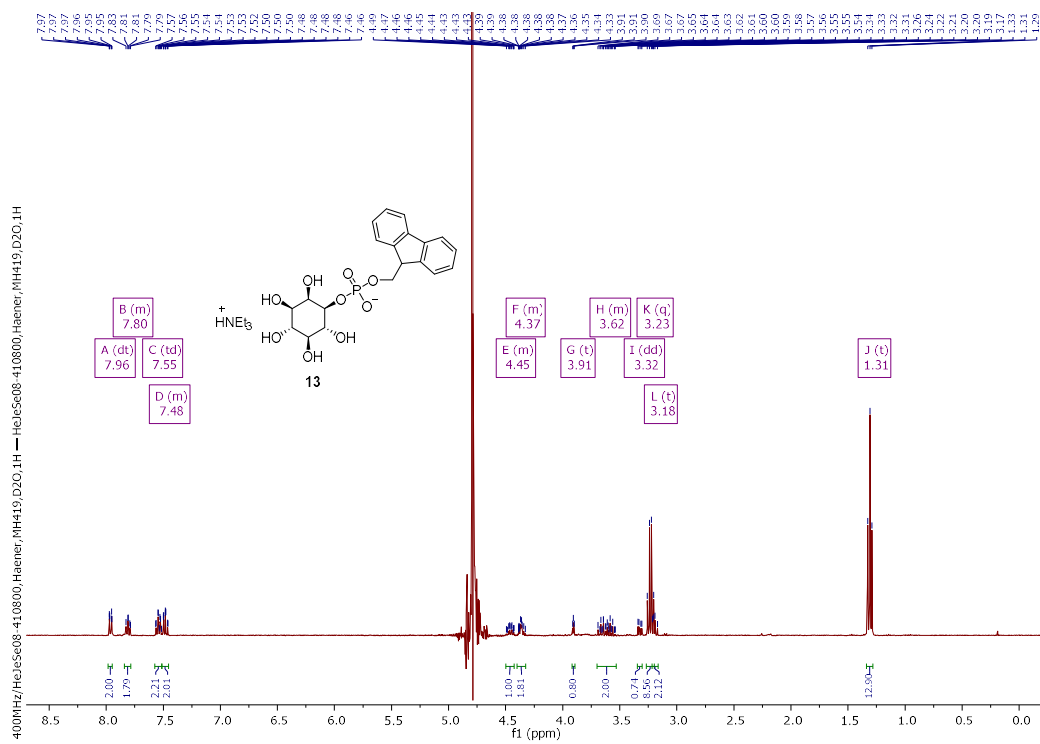
Supplementary Figure 44 ^1H -NMR of **12**.



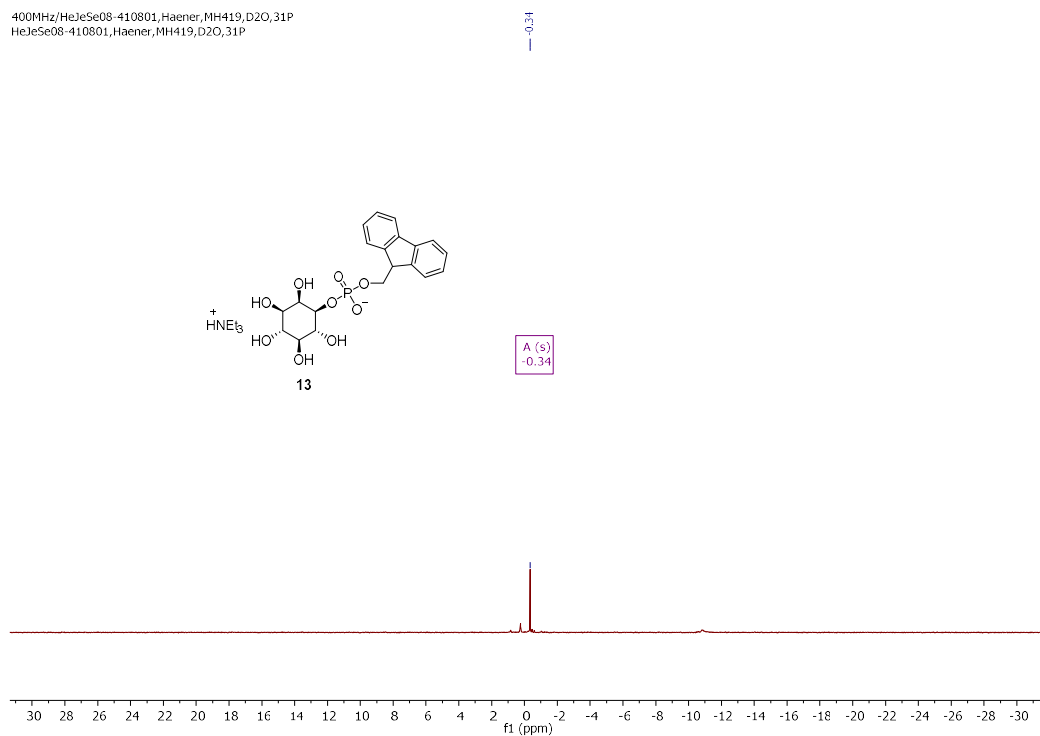
Supplementary Figure 45 ³¹P-NMR of **12**.



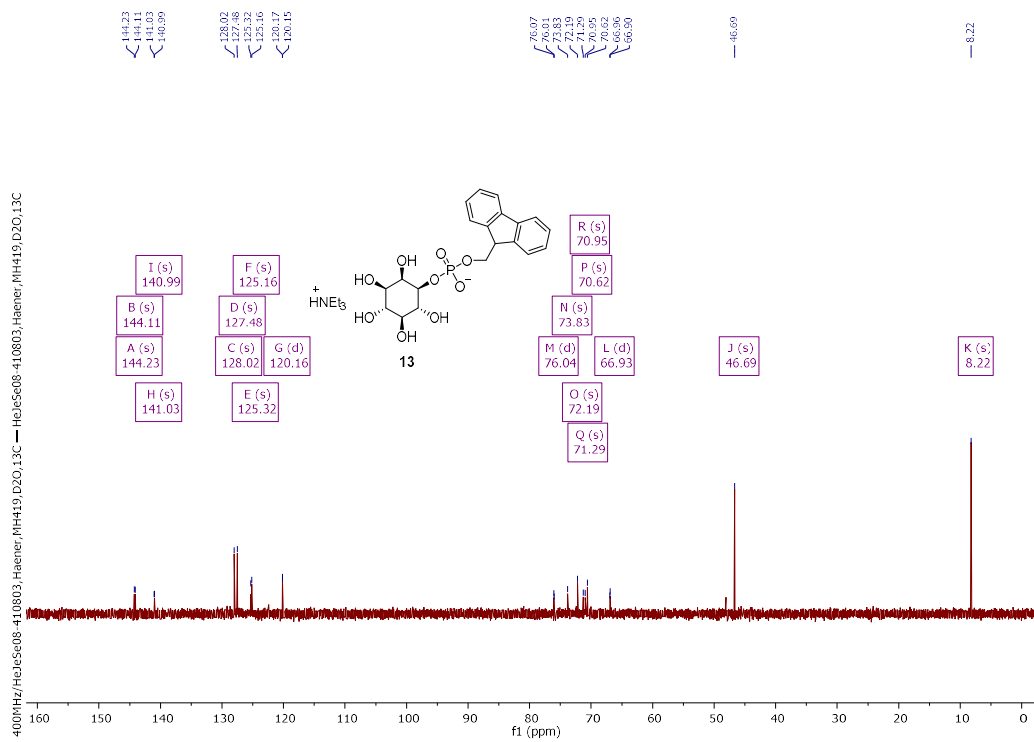
Supplementary Figure 46 ¹³C-NMR of **12**.



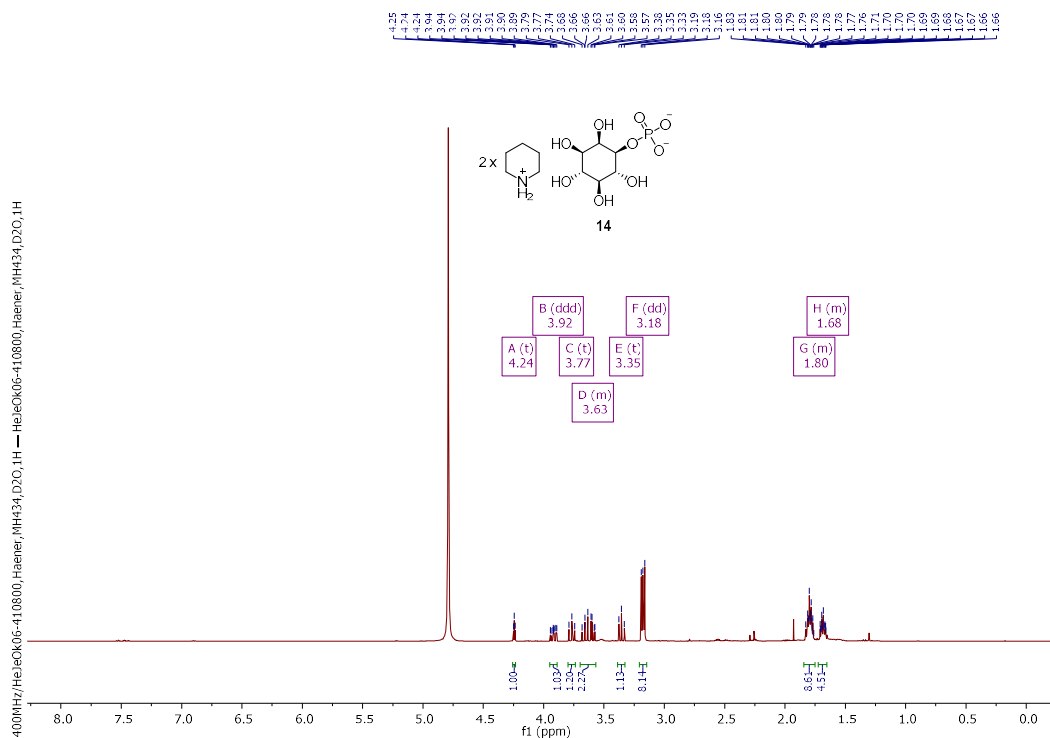
Supplementary Figure 47 ^1H -NMR of **13**.



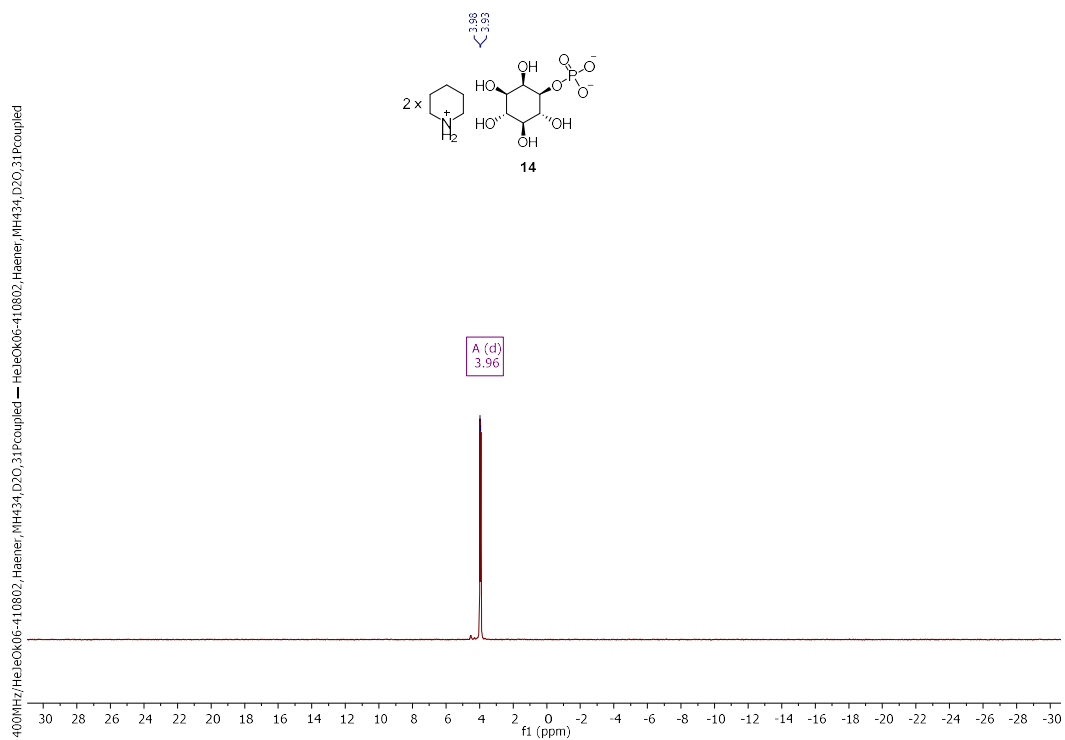
Supplementary Figure 48 $^{31}\text{P}\{^1\text{H}\}$ -NMR of **13**.



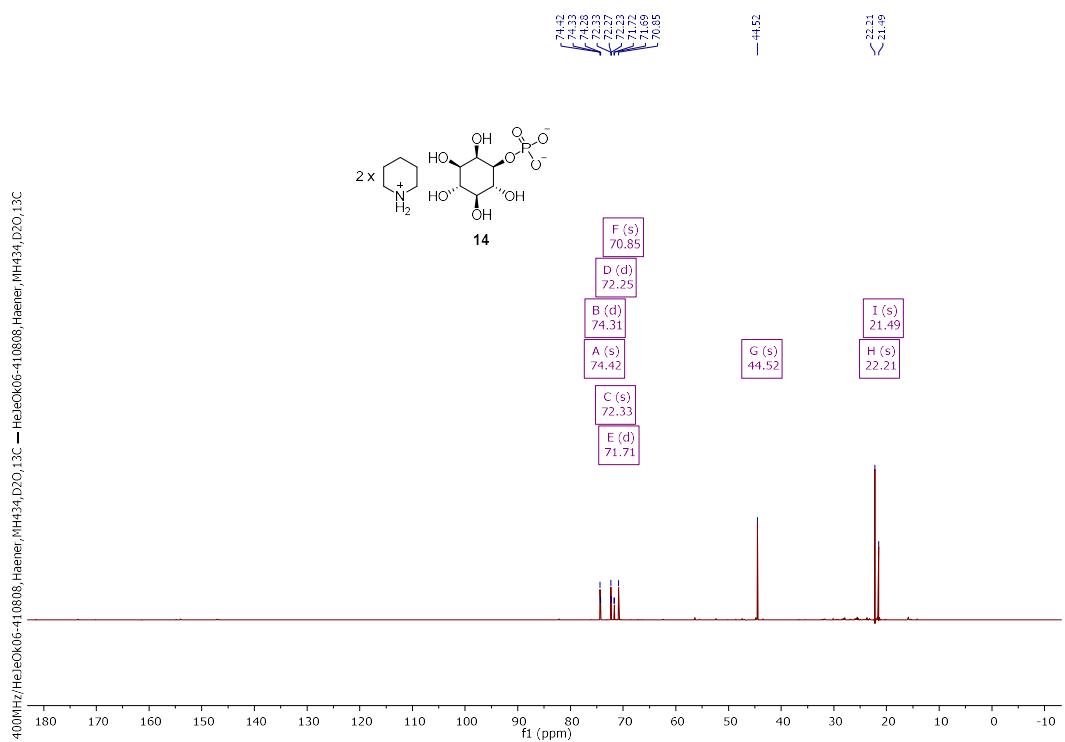
Supplementary Figure 49 ^{13}C -NMR of **13**.



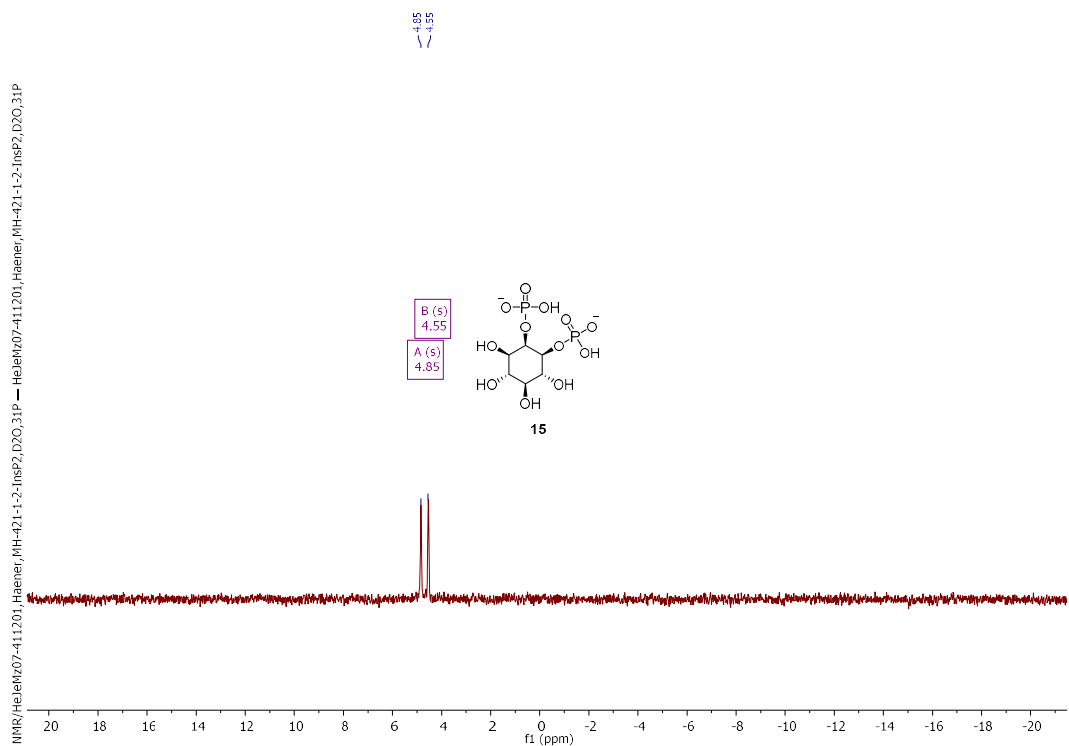
Supplementary Figure 50 ^1H -NMR of **14**.



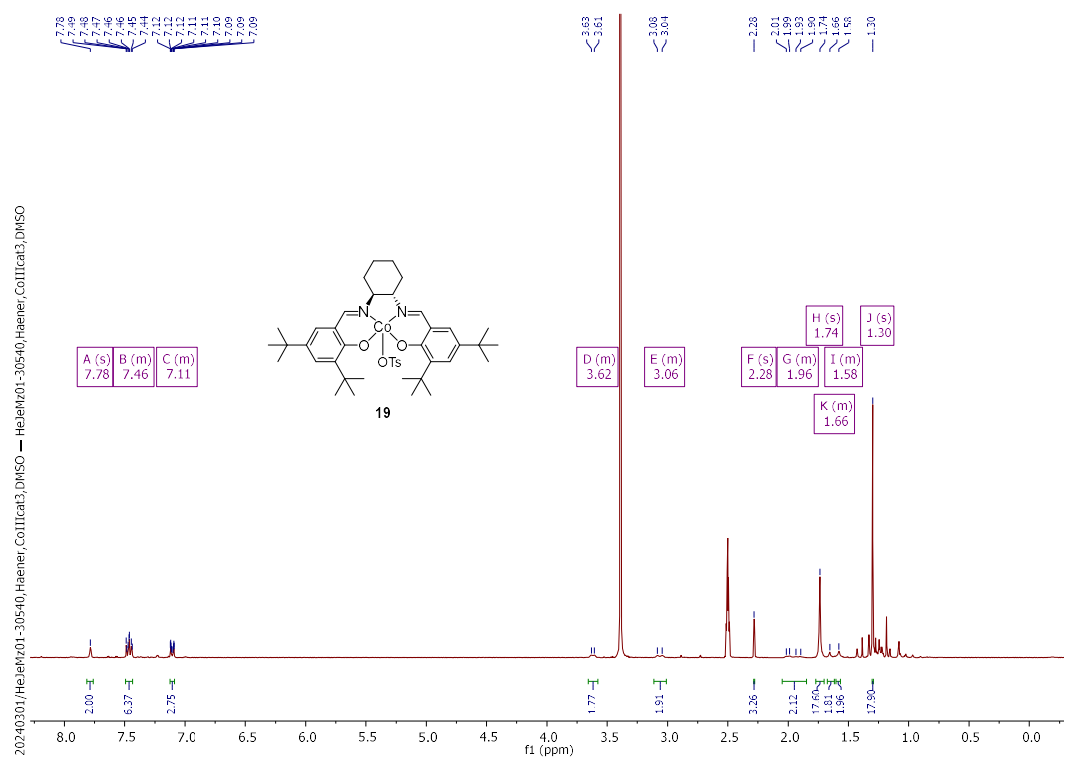
Supplementary Figure 51 ^{31}P -NMR of **14**.



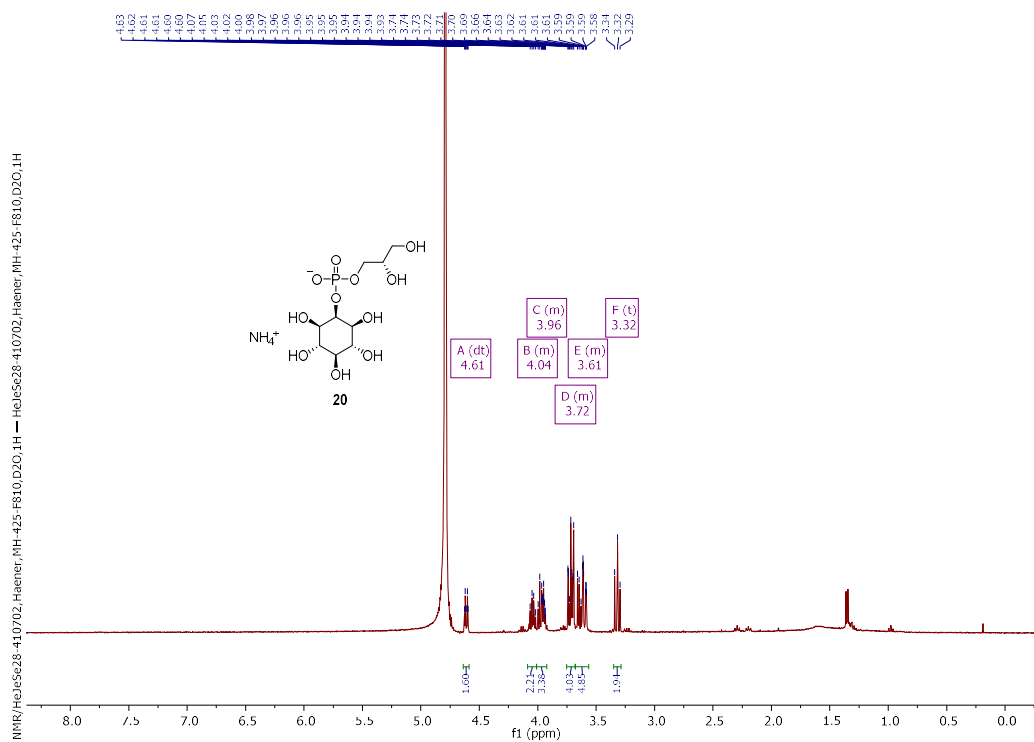
Supplementary Figure 52 ^{13}C -NMR of **14**.



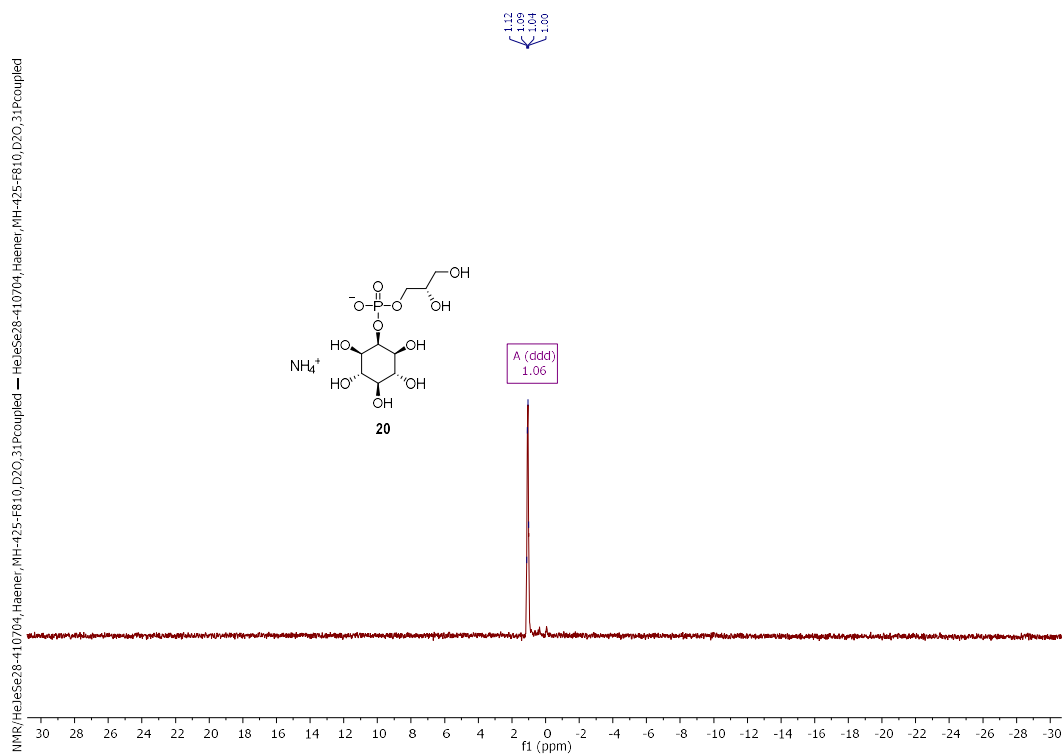
Supplementary Figure 53 $^{31}\text{P}\{^1\text{H}\}$ -NMR of **15**.



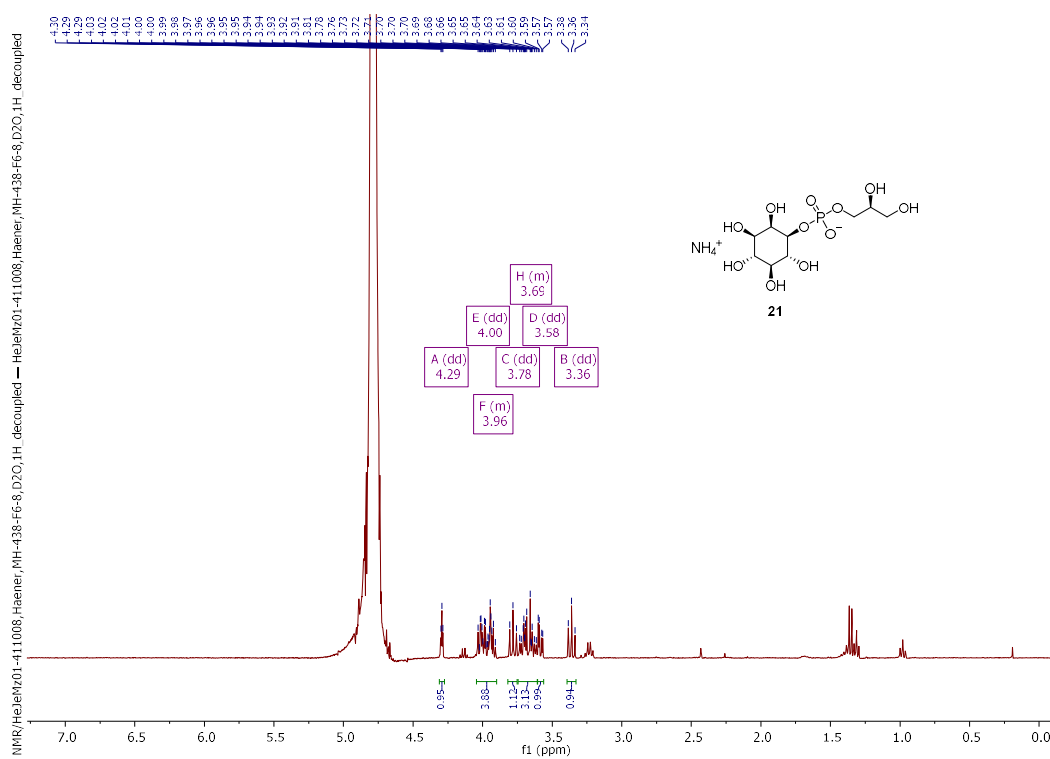
Supplementary Figure 54 ^1H -NMR of **19**.



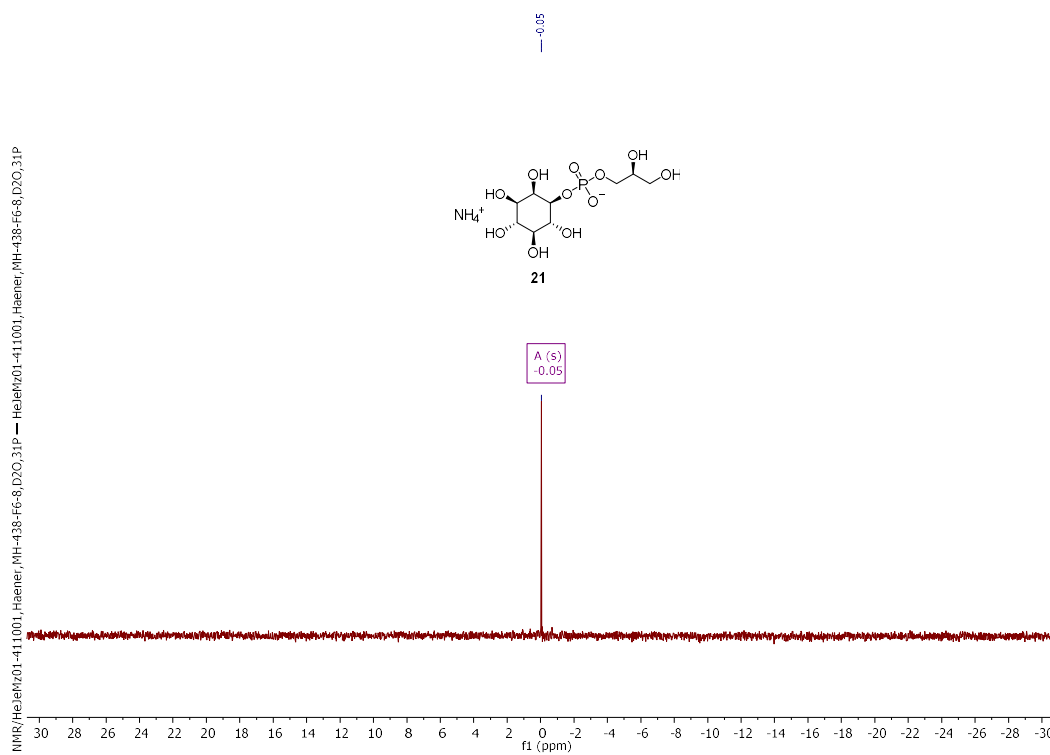
Supplementary Figure 55 ^1H -NMR of **20**.



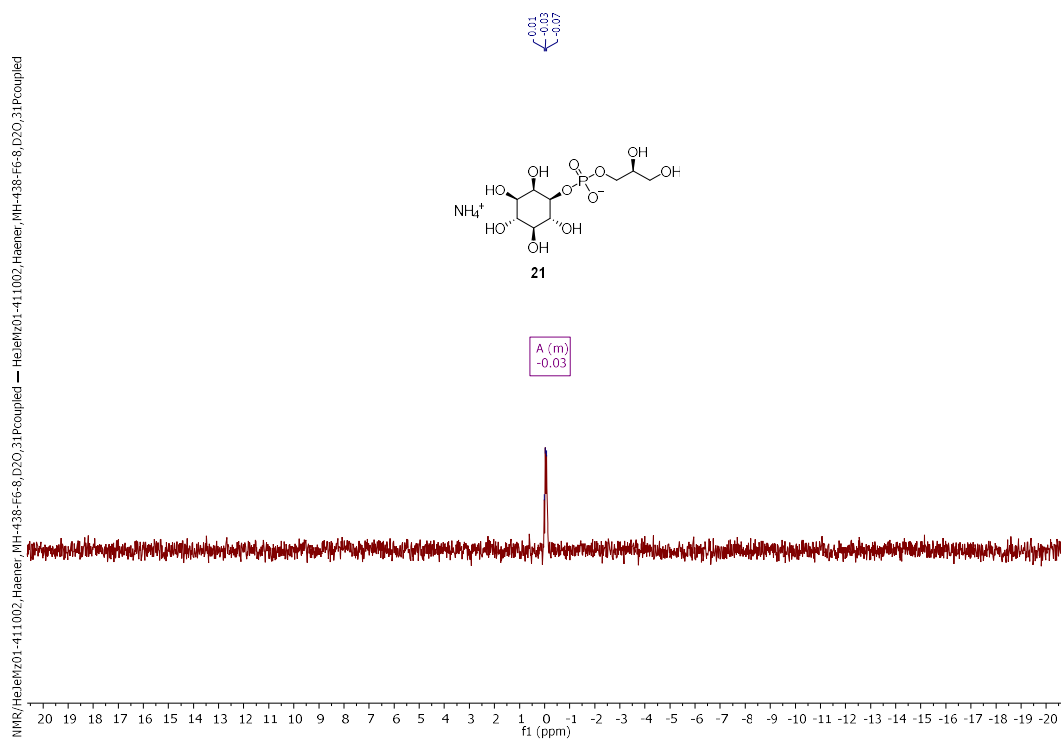
Supplementary Figure 56 ^{31}P -NMR of **20**.



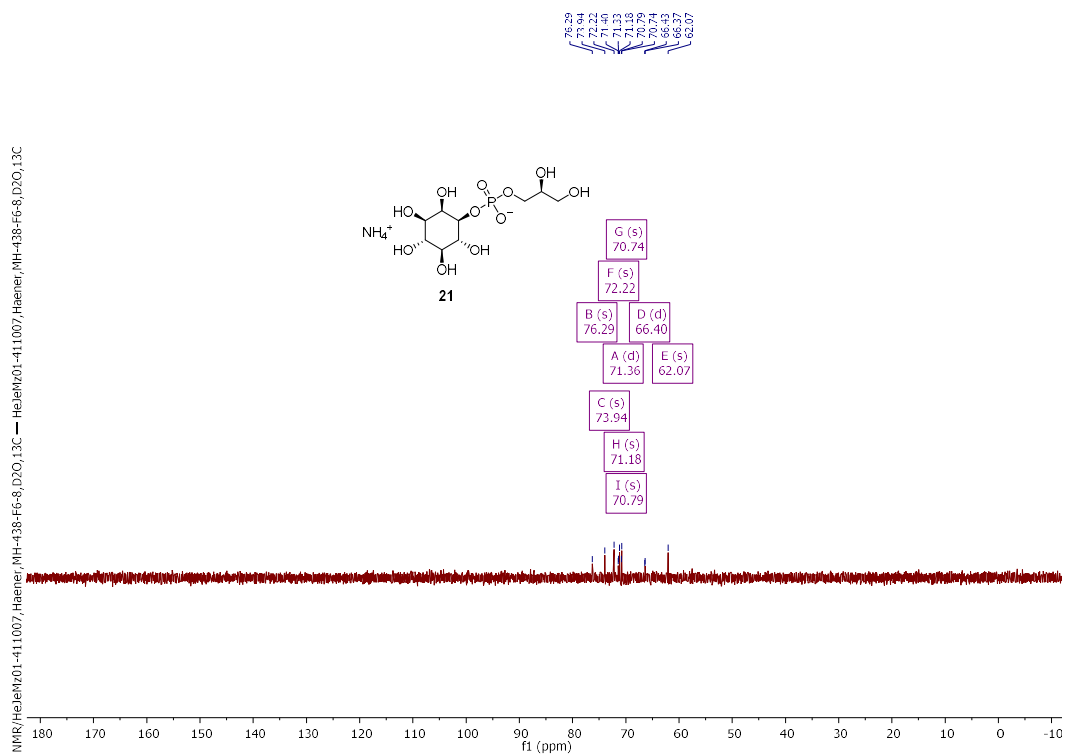
Supplementary Figure 57 ^1H -NMR of **21**.



Supplementary Figure 58 $^{31}\text{P}\{^1\text{H}\}$ -NMR of **21**.



Supplementary Figure 59 ^{31}P -NMR of **21**.



Supplementary Figure 60 ^{13}C -NMR of **21**.